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OWEN F. BURGER

1885–1928

GEORGE F. WEBER

Owen Francis Burger, plant pathologist of the Florida Agricultural Experiment Station and State Plant Board since 1920, died January 26, 1928, in the Good Samaritan Hospital at West Palm Beach, from injuries received in an automobile collision Monday evening, January 23.

He was born at Freeland, Pennsylvania, June 8, 1885, the youngest of a large family. At an early age he was left fatherless.

During his boyhood he attended school at Noblesville, Ind., where he graduated from high school in 1904. He entered the University of Indiana in the fall of 1905 and received an A. B. degree from that institution in 1909, having majored in the botanical sciences. Immediately after graduation, he took up his duties as an assistant to the plant pathologist of the Florida Experiment Station. He pursued graduate work in the University of Florida while working as an assistant and received an M.S. from the University in 1911.

While at the Florida Experiment Station, he was assisting Dr. H. S. Fawcett, from whom was undoubtedly received the inspiration and desire to make mycology and plant pathology a life work. At this time in Dr. Burger's career there developed the desire to serve that was so profoundly manifest in later years.

After receiving the M.S. degree he was made assistant plant pathologist of the Florida Experiment Station. He held this position for two years, leaving in the fall of 1913 in order to pursue graduate work at Harvard University. During 1913–14 he was a University scholar at Harvard and during the following two years was a Priscilla Clark Hodges scholar in the same institution. He was granted an M.S. degree from Harvard in 1915 and a D.Sc. in June, 1916.

Dr. Burger was appointed instructor in plant pathology at the Citrus Experiment Station, University of California, in 1916, remaining until 1918, when he was made a pathologist in the United States Department of Agriculture, at Washington, D. C.

While in the Federal Bureau of Plant Industry Dr. Burger had charge of the then recently organized work on market pathology of fruits. For this work his broad experience, wide interests, and ability as a mixer made him particularly well qualified. He was equally at home among the pathologists, the food products inspectors, the wholesalers and fruit brokers, and the freight handlers on the docks. This experience broadened his outlook with respect to the field of plant pathology and thus influenced his development of the department of plant pathology in Florida. In December, 1920, he was made plant pathologist of the Florida Experiment Station in which capacity he was acting at the time of his death.

Dr. Burger readily qualified in that honorable group known as self-made men. His early education was obtained only by means of an unrelenting struggle. He earned his way as he went. through college and during his graduate work.

While in college he was popular and was one of the "gang" that was often suspected of indulging in college pranks. His qualities of good fellowship made him equally well liked among the American phytopathologists, where, as every reader of this journal knows, a spirit of comradeship generally prevails. A note from one of his former colleagues includes the following statement: "My own clearest recollections of O. F. and my best memories of him are, I must confess, not associated with our long discussions of the field and possibilities of market pathology, but with certain lively hours at botanical dinners when half a dozen carefully chosen spirits gathered around a table at a discreet distance from the officers' or speakers' table; and Burger was, of course, the center of the fun." He met all comers man to man, and his six-foot-one height and more-than-two-hundred-pounds weight made him an adversary to be considered.

He was married July 22, 1916, to Helen Sanborn Lothrop, of Boston, Massachusetts, who survives him. His home life was most pleasant. He was a loyal, thoughtful, and devoted husband. His wife was his best companion; they lived for each other, she being at his bedside when he passed to the great beyond. As a man Dr. Burger measured up in full. It is to be regretted that one so big-hearted and lovable could not be spared.

Dr. Burger was a devout Christian. In his early life he attended services regularly and was especially interested in young peoples' organizations. He was a member of the Episcopal Church.

Dr. Burger will be remembered by the majority of Florida people for what he did to improve agriculture in this state. His basic education was broad and liberally grounded in the arts and sciences. Practical work in the field and laboratory, conducted parallel with his graduate studies, acquainted him with the problems of the man of the soil. His most important

work was with the citrus grower. He conducted experiments on blue mold decay and stem-end rot of citrus.

A large share of his efforts in the past few years were devoted to improvement in methods of disease control. He took great interest in the functioning of the State Plant Board. He cooperated in formulating and putting into effect state and federal quarantine rules and regulations. He assisted in acquainting railroad employees with the types and amount of disease occurring in transit. Sectional meetings in the state, held for the purpose of conveying recent information from the laboratory directly to the grower, usually found him a principal speaker on the program. His fund of knowledge and his dynamic personality were his outstanding qualities as a teacher and platform speaker. He travelled extensively in the state, visiting farms, groves, and packing houses.

He was alone in the Department of Plant Pathology when he assumed his duties in 1920. It was not long, however, until he was convinced that the need for progress was so great and the problems so numerous that he must have aid. In surrounding himself with assistants he demonstrated his broad vision by selecting men fully prepared to conduct research work. He desired men who were mature, even though inexperienced. He selected them from the larger institutions where leaders in plant pathology were located.

At the time of his death his staff consisted of twelve trained men scattered over the state in the sections where they were most needed, all doing more or less individual research toward a common end, the assistance of the producer and consumer. He demonstrated his leadership and ability as an organizer by developing a large and smoothly functioning department. His ideas of service and his regard for the truth have been instilled into every one of his assistants. His breadth of vision, personality, sense of duty, and ambition were four reasons for his success. His ability as a scientist was equalled, or possibly surpassed, by qualities of leadership and organization which contributed so largely to his success as a director of the numerous activities under his supervision.

The preliminary work of selection of personnel and the organization of projects in plant pathology was almost completed by Dr. Burger. The periods of anxiety and doubt were past. He was beginning to see the end of the formative period and about to enter into the productive field. He was taken from his desk when the load was beginning to lighten. Under ordinary circumstances he would have enjoyed a score of years of productive work in the state with due recognition from the nation.

Dr. Burger was a fellow of the American Association for Advancement of Science and a member of the following: American Phytopathological

Society, Botanical Society of America, American Agricultural History Society, British Mycological Society, Florida Entomological Society, Florida Horticultural Society, New England Botanical Club, Phi Kappa Phi (local treasurer), Sigma Xi (local vice-president), the Masonic Order, and Kiwanis Club.

The following is a chronological list of Dr. Burger's scientific publications:

A gum-inducing *Diplodia* of peach and orange. Mycologia **3**: 51–53. 1911 (with H. S. Fawcett).

A variety of *Cladosporium herbarum* on *Citrus aurantium* in Florida. Phytopath. **1**: 64–66. 1911 (with H. S. Fawcett).

Tomato rust. Fla. Agr. Exp. Sta. Press Bul. 207. 1913.

A bacterial rot of cucumbers. Phytopath. **3**: 169–170. 1913.

Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121: 97–109. 1914.

Cucumber and cantaloupe blight. Fla. Agr. Exp. Sta. Press Bul. 221. 1916.

Observations on a fungus enemy of the walnut aphis in southern California. Jour. Econ. Entom. **2**: 278–288. 1918 (with A. F. Swain).

Sexuality in *Cunninghamella*. Bot. Gaz. **68**: 134–146. 1919.

Decay in citrus fruits during transportation. Cal. State Dept. Agr. **9**: 365–370. 1920.

Decay of citrus fruits in transit. Fla. Agr. Exp. Sta. Press Bul. 322. 1920.

Variations in *Colletotrichum gloeosporioides*. Jour. Agr. Res. **20**: 723–736. 1921.

Watermelon diseases. Quart. Bul. Fla. St. Plant Board **5**: 131–138. 1921.

Peronspora disease of tobacco. Quart. Bul. Fla. St. Plant Board **5**: 163–167. 1921 (with H. C. Parham).

Red rot of sugar cane. Fla. Agr. Exp. Sta. Press Bul. 334. 1922.

Spraying to control melanose. Fla. Agr. Exp. Sta. Press Bul. 335. 1922 (with E. F. DeBusk).

Spray schedule for peaches. Fla. Agr. Exp. Sta. Press Bul. 336. 1922 (with J. R. Watson).

Some sweet potato diseases. Quart. Bul. Fla. St. Plant Board **6**: 71–76. 1922 (with A. C. Brown).

Green spotting of citrus fruits. Fla. Agr. Exp. Sta. Press Bul. 342. 1922 (with E. F. DeBusk).

Black rot of oranges. Fla. Agr. Exp. Sta. Press Bul. 343. 1922 (with Wm. Gomme).

Preliminary report on control of melanose and preparing bordeaux-oil. Fla. Agr. Exp. Sta. Bul. 167: 123–140. 1923 (with E. F. DeBusk and W. R. Briggs).

Florida citrus diseases. Fla. Agr. Exp. Sta. Bul. (in press) (with A. S. Rhoads and E. F. DeBusk).

Numerous brief and more popular articles have appeared in reports of the plant pathologist of the Florida Agricultural Experiment Station, in Florida Horticultural Society reports, Citrus Industry, Florida Grower, and elsewhere.

THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS TRITICI IN A CROSS BETWEEN TWO VARIETIES OF TRITICUM VULGARE[1]

C. H. Goulden, K. W. Neatby, and J. N. Welsh[2]

Wheat varieties that are susceptible to certain physiologic forms of *Puccinia graminis tritici* in the seedling stage often show very high resistance under field conditions. Hayes *et al.* (5) describe the inheritance of rust resistance in a cross of Marquis × Iumillo with Marquis and show that the Marquis × Iumillo resistance shown in the field is dependent on at least two genetic factors which are inherited apparently independently from a single factor for immunity from certain physiologic forms under greenhouse conditions. In this instance the resistance shown in the field is not necessarily a different type of resistance from that shown in the greenhouse. Physiologic forms other than the group to which the Marquis × Iumillo parent is immune were evidently present in the field, and the results indicate that a different set of factors were operating for resistance to these forms. Aamodt (1) points out that the reaction to particular physiologic forms shown by seedling plants in the greenhouse may not necessarily be a criterion of the reaction in the field and in addition gives an excellent review of published data on this phase of rust investigations. Aamodt states that, "Immunity or a high type of resistance can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field. Moderate resistance, however, must be differentiated under field conditions in order to have an accurate determination of the reaction of the plant." The question as to the value of a greenhouse test for resistance is therefore left somewhat in doubt. In order to answer the question satisfactorily it is evident that tests conducted on plants approaching maturity must be carried out under conditions such that the physiologic forms may be controlled. The lack of agreement between field and greenhouse results may be due on the one hand to additional rust forms that are present in the field and on the other hand to the expression in plants approaching ma-

[1] The writers wish to acknowledge very gratefully the assistance of Dr. Margaret Newton and Messrs. T. Johnson and A. M. Brown in the physiologic form work. Mr. R. F. Peterson, student assistant gave valuable assistance throughout the entire study.

[2] Senior Cerealist and Cerealists at the Dominion Rust Research Laboratory, Winnipeg, Canada. Contribution from the Cereal Division, Dominion Experimental Farms Branch, Ottawa.

turity of a type of resistance that cannot be detected in the seedling stage. An increasing resistance as the plants of certain strains mature does not necessarily indicate an increase in the physiologic resistance shown in the seedling stage but perhaps merely the superimposing of a mature plant type of resistance which may or may not be physiological.

The present study was undertaken in order to obtain further information on the inheritance of high degrees of both seedling and field resistance, and particularly on the relation between the two types of resistance.

PARENT MATERIAL

The resistant parent in the cross studied is known as H–44–24 and was produced by McFadden (7) by crossing Yaroslav emmer with Marquis wheat. This strain has been almost entirely free from rust during the past three seasons in tests conducted by the Botany Division at all of the experimental stations in Western Canada. Occasionally, small pustules may be found just above the nodes but it is quite certain that damage from rust is negligible. Agronomically, H–44–24 is probably not of much value since it is somewhat short and weak in the straw, has rather persistent glumes, and a slightly brittle rachis. As to the milling quality of the grain not much information is yet available but one test has been made

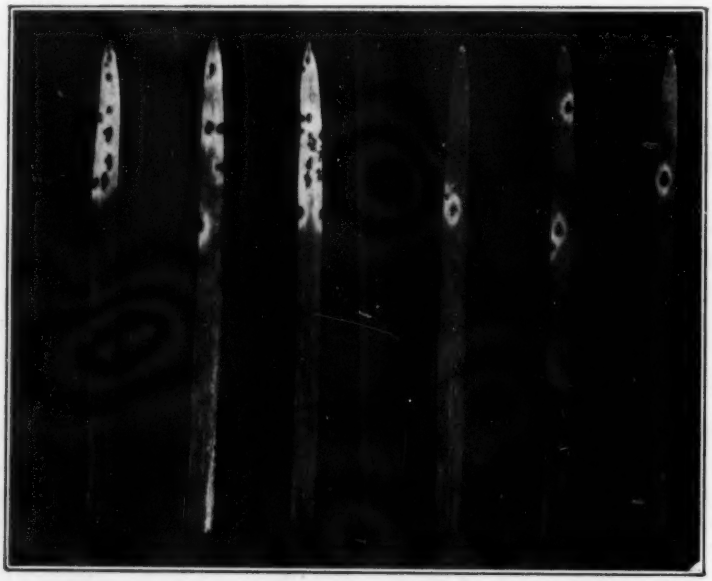

FIG. 1. Seedling reactions of Marquis (left) and H–44–24 (right) to physiologic form 21.

at the Cereal Division Milling and Baking Laboratory at Ottawa. From the standpoint of loaf volume, texture, and color of bread, the wheat was quite satisfactory. The crust was somewhat durum-like in character.

Seedling tests of H–44–24 for rust resistance to seven physiologic forms have been conducted by Newton and Johnson (8), and table 1 is an excerpt from their publication comparing the reactions of H–44–24 and Marquis.

TABLE 1.—*The reaction of H–44–24 and Marquis, common spring wheats, to seven physiologic forms of Puccinia graminis tritici*

Varieties	Host reaction to physiologic forms						
	21	29	30	32	34	36	+[a]
H–44–24, R.L. 229	2+	2+	3–	—	—	0; 1	1
Marquis, R.L. 84	4	4–	4	4=	4–	4	2–

[a] An unidentified form.

The reactions of H–44–24 and Marquis to forms 21 and 36 are shown in figures 1 and 2.

Marquis was chosen as the other parent in the cross owing to its desirability, with the exception of rust reaction, for conditions in Western Canada.

FIG. 2. Seedling reactions of Marquis (left) and H–44–24 (right) to physiologic form 36.

FIG. 3. Seedling reaction of F_1 plants of H–44–24 × Marquis to physiologic form 21.

FIG. 4. Seedling reaction of F_1 plants of H–44–24 × Marquis to physiologic form 36.

THE F_1 GENERATION

The first generation plants were all grown in the greenhouse during the winter of 1925–26. Seedlings of F_1 plants were tested to the two forms 21 and 36 with results as shown in figures 3 and 4.

The moderate resistance of H–44–24 to form 21 is recessive in the F_1 while the high resistance to form 36 is dominant. As will be shown later this is supported by data on the seedling reactions of the F_3 families.

An interesting feature of the resistance of F_1 plants was observed when these were inoculated with form 21 at different stages of maturity. The results, as given in table 2, and illustrated in figure 5, show that seedling

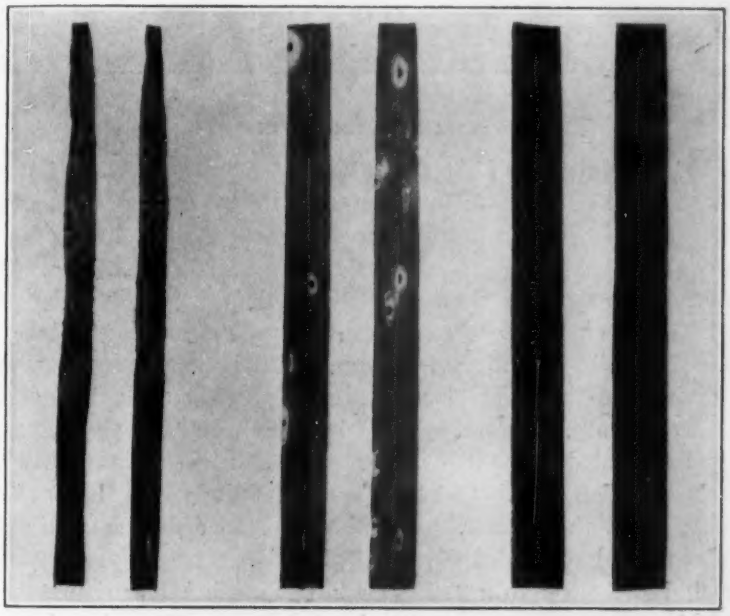

FIG. 5. Leaves from F_1 plants of H–44–24 × Marquis inoculated with physiologic form 21 at different stages of maturity. Left, seedling stage; centre, shot blade; right, fully headed.

susceptibility in this case changes very quickly to moderate resistance and by the time the plants are fully headed they show almost complete resistance. As evident from the table, each test was conducted in duplicate. This result indicated the possibility of the presence of factors for mature plant resistance which completely covered up the susceptibility evident only in the seedling stage.

TABLE 2.—*Rust reaction of F_1 plants of H–44–24 × Marquis at different stages of maturity, to physiologic form 21*

Date planted	Plot no.	Rust reaction on	
		Leaf	Culm
June 3	10	3 ++	—
	9	3 ++	—
May 20	8	2 ±	2
	7	2 ±	3c
May 6	6	2 –	3 ± c
	5	2 –	3 – c
April 22	4	1 –	1 – 2
	3	1	1 ±
April 8	2	0	1 –
	1	0	1 –

THE F_2 GENERATION

In 1926 a population of about 5000 F_2 plants was grown in the experimental field of the Dominion Rust Research Laboratory at Winnipeg, and an artificial rust epidemic was produced with the seven physiologic forms listed in table 1. The epidemic was a little late getting started, but sufficient rust was present to show that segregation was taking place in the mature plants of the F_2 population and that resistance was partially dominant over susceptibility. A random sample of 1054 plants was taken and the plants divided as they were harvested into three groups, R (resistant), SR (semi-resistant), and S (susceptible), the reaction being determined entirely from rust development on the stems and leaf sheaths. According to this classification the H–44–24 fell quite definitely into the R class and the Marquis into the S class. Assuming resistance dominant, the results are as given in table 3 and fitted to a 3 : 1 ratio.

The fit is not good to a 3 : 1 ratio but is much better than to a 15 : 1 ratio. This result was somewhat to be expected as the epidemic was not severe enough to differentiate between S and SR types. The numbers obtained in the three classes were R–301, SR–555, and S–198. The deficiency in the S class seems to have been added to the SR class and this is borne out by results on the F_3 families.

GREENHOUSE TESTS ON F_3 GENERATION FAMILIES

During the winter of 1926–27 portions of each F_3 family were tested to forms 36 and 21. The parent reactions shown in figures 1 and 2 have

TABLE 3.—*Segregation in the field of the F_2 population of H–44–24 × Marquis for resistance and susceptibility, and calculation of goodness of fit to a 3:1 ratio*

Groups	Actual	Theoretical (3:1)	Dev.	P.E.	$\frac{\text{Dev.}}{\text{P.E.}}$
Resistant and semi-resistant	856	790.5	65.5	9.48	6.91
Susceptible	198	273.5			

already been described. To form 36 the F_3 plants showed amounts of resistance varying from the resistance of H–44–24 to the susceptibility of Marquis. An intermediate type of resistance was also observed which was uniform in a number of lines. Three distinct reaction types are shown in figure 6. Since lines were obtained breeding true for the three distinct

FIG. 6. Three degrees of resistance shown by F_3 lines of H–44–24 × Marquis to physiologic form 36. Left susceptible; centre, semi-resistant; right, resistant.

types, two factors governing the resistance to form 36 are indicated and this is borne out by a summary of the results from 745 F_3 families, the total number successfully tested to form 36. The families were classified according to their greenhouse reaction as homozygous susceptible, segregating, and homozygous resistant, and the data arranged as in table 4.

TABLE 4.—*Numbers of F_2 families of H–44–24 × Marquis classified by their greenhouse reaction as homozygous susceptible, segregating, and homozygous resistant to physiologic form 36.*

F_1 plant number	Number of F_2 families			Total no. families
	Homozygous susceptible	Segregating	Homozygous resistant	
550	0	38	12	50
538	2	44	8	54
535	5	50	13	68
534	7	31	9	47
543	13	29	10	52
541	6	36	17	59
537	3	40	4	47
551	4	13	5	22
548	1	23	1	25
533	0	11	1	12
542	1	34	11	46
545	2	50	13	65
546	0	25	11	36
549	1	19	5	25
539	2	7	1	10
540	2	36	10	48
547	0	23	4	27
544	0	39	13	52
Totals	49	548	148	745

It is evident that the ratio of homozygous susceptible families to the remainder is close to 1 : 15. The actual ratio is 49 : 696, and the theoretical 1 : 15 is 46.6 : 698.4. The deviation 2.4, divided by the probable error 4.46, gives 0.54, showing a very close fit.

The factor relations for resistance to form 36 in this cross are probably as illustrated in diagram 1.

In obtaining the actual data it was often very difficult to distinguish between homozygous R and SR types, so these have been added together in the summarized results as shown in table 5. (It was much easier to distinguish families segregating for R and SR.) The theoretical ratio for the three types is therefore 3 homozygous resistant, 12 segregating, and 1 homozygous susceptible. The comparison between actual and theoretical numbers is also given in table 5.

For three classes P = about 0.6 for $X^2 = 0.81$, Pearson (9), and Fisher (2). The fit is therefore very good.

The F_3 lines tested to form 21 were classified as homozygous resistant, segregating, and homozygous susceptible. By resistance in this case is

DIAGRAM 1. *Probable factor relations in the cross H–44–24 × Marquis for resistance in the seedling stage to form 36*

Parents ..... H–44–24 – $R_1R_1R_2R_2$

Marquis – $r_1r_1r_2r_2$

F_1 ..... $R_1r_1R_2r_2$

F_2	Theoretical frequency of genotypes	Genotypes	Phenotypes	Segregation in F_3
	1	RRRR	R	R
	2	RRRr	R	3R + 1SR
	2	RrRR	R	3R + 1SR
	4	RrRr	R	9R + 6SR + 1S
	1	rrRR	SR	SR
	1	RRrr	SR	SR
	2	Rrrr	SR	3SR + 1S
	2	rrRr	SR	3SR + 1S
	1	rrrr	S	S

TABLE 5.—*Numbers of F_3 families, homozygous resistant, segregating, and homozygous susceptible, to form 36, and calculation of goodness of fit to a theoretical 3:12:1 ratio*

Groups	Actual	Theor. (3:12:1)	$(A-T)^2/T$
Hom. R + SR	148	139.7	.49
Segregating	548	558.7	.20
Hom. S	49	46.6	.12
Totals	745	745.	.81 = X^2

meant the moderate resistance shown by the parent H–44–24 to form 21. The parental types recovered in the F_3 families are similar to those shown in figure 1. Aamodt (1) has referred to the reaction of H–44–24 to form 21 as moderately susceptible. This is perhaps a difference in terminology only. The term resistant was used in our case partly for convenience and partly on account of the hypersensitive areas around the pustules which led Newton and Johnson in 1927 to classify the reaction as 2+. Tests were conducted again in the fall of 1927 using form 21 on H–44–24 and many leaves showed a large number of small pustules without distinct hypersensitive areas. However, during the winter of 1926–27, when the F_3 families were tested, H–44–24 was used as a check and the reaction was consistently that shown in figure 1.

As might be expected, considerable difficulty was encountered in classifying the F_3 families but an attempt was made to make as careful a de-

termination as possible of the families breeding true for the reactions typical of the parents. The results are as shown in table 6.

TABLE 6.—*Numbers of F_3 families of H–44–24 × Marquis classified by their greenhouse reactions as homozygous susceptible, segregating, and homozygous resistant, to form 21*

F_1 plant no.	Number of F_3 families			Total no. families
	Homozygous susceptible	Segregating	Homozygous resistant	
550	7	9	4	20
535	6	9	4	19
534	10	26	5	41
543	12	19	5	36
541	31	16	7	54
537	9	33	8	50
551	30	39	5	74
548	14	38	4	56
533	16	27	2	45
542	16	26	9	51
545	14	40	8	62
546	23	27	1	51
549	14	6	2	22
539	17	26	7	50
540	18	33	2	53
547	10	24	4	38
544	17	42	2	61
Totals	264	440	79	783

Assuming susceptibility to the dominant as indicated by the F_1 reaction, the ratio obtained from the totals in table 6 does not give a good fit to either a 3:1 or 15:1 ratio. The goodness of fit is shown in table 7.

Since the fit is closest to a 15:1 ratio, it seems reasonable to assume a difference of two pairs of factors between the parents in this cross for moderate resistance to physiologic form 21.

GREENHOUSE TESTS ON F_4 GENERATION FAMILIES

Previous studies by other investigators, Puttick (10), Harrington and Aamodt (3), and Hayes and Aamodt (4), have demonstrated that the resistance of two varieties which react reciprocally to two physiologic forms of rust could be combined in a single variety. It is conceivable that in certain cases the factors governing high resistance to one or more forms may also govern a reaction to one or more other forms and the latter may be only moderate resistance or perhaps susceptibility. In the present study some indications have been obtained that this is actually the case. Fourteen F_4 lines which were known to be breeding true for high resistance to

TABLE 7.—*Numbers of F_3 families falling into the two classes, homozygous susceptible and segregating, and homozygous resistant, and calculation of the goodness of fit to 3:1 and 15:1 ratios*

Ratio	Actual		Theoretical		Dev.	P.E.	Dev./P.E.
	Susc. and segregating	Resistant	Susc. and segregating	Resistant			
3:1	704	79	587.2	195.8	116.8	8.17	14.3
15:1	704	79	734.1	48.9	30.1	4.57	6.3

form 36 and moderate resistance to form 21 were tested in the seedling stage to seven forms, 9, 14, 15, 17, 21, 34, and 36. These all gave reactions similar to those of the H–44–24 parent. Eight F_4 lines breeding true for susceptibility to forms 21 and 36 were also tested to forms 9, 14, 15, 17, and 34. These all gave reactions similar to the Marquis parent including high resistance to form 14. The results are summarized in table 8.

TABLE 8.—*Reaction of twenty-two selected F_3 lines of H–44–24 × Marquis to seven physiologic forms of P. graminis tritici*

Field reaction	Strain no.	Physiologic forms						
		9	14	15	17	21	34	36
Susc.	7	2 3–	2 3	3+-	2+ 3–	2 3	2 3	
	204	2	2 3	3+-	2	2 3–	2 3	1
	293	2 3–	2 3	3+	2+ 3–	2 3	3– 3	1
	456	2+ 3–	2–: 1	3+-	3–	2 3	2 3	1
	572	3	2 3	3	2+	2 3	2 3	1
	587	3	2 3	3+	2+	2 3	2 3	1
	885	2+ 3–	2 3–	2+ 3	2	2 3	2 3	1
	937	3+	2 3	3+	3–	3–	3–	1
Res.	128	2 3–	2 3	3+	2 3	2 3	2 3	1–
	386	2 3+	2 3	3+	3	2 3	3+-	1–
	725	2 3		3+	2 3	2 3	2 3	1–
	880	2 3	2 3	3+	2 3	2 3	2 3	1
	888		2 3	3+-	3	2 3	3	1–
	903	2 3	2 3+	3+-	2 3	2 3	2 3	1–
Res.	99	4	1–	4	3+–		3+	
	148	3+	1	3+	3+		3+	
	179	3+	1	4	3+		3+	
	208	4	1	4	4		4	
	221		1	4+	3+		3+	
	331	3+	1	3+-	3+		3+	
	450	3 4–	1+	3+	3		3+	
	875	4	1	4	4–		3+	

In this test only those lines were used which were either homozygous resistant or susceptible to both forms, 21 and 36, so it was impossible to determine whether the factors governing resistance to 21 or those governing resistance to 36 were also responsible or closely linked to factors governing resistance to the other forms. A further test was conducted on a group of twelve miscellaneous strains which were varied in their reactions to both forms. The results of this test are presented in table 9. It will

TABLE 9.—*Seedling reactions*[a] *of twelve miscellaneous* F_4 *lines of H–44–24 × Marquis, to physiologic forms 21, 14, 17, and 36*

Strain no.	Physiologic forms			
	21	14	17	36
105	Hom. MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
125	Seg. S + MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
248	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R
268	Hom. S	Hom. R	Seg. S + MR	Hom. R
323		Hom. R	Seg. S + MR	Seg. MS + R
349	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R
374	Hom. S	Hom. R	Hom. S	Seg. MS + R
431	Seg. S + MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
490	Hom. S	Hom. R	Hom. S	Seg. MS + R
503	Seg. S + MR	Seg. MR + R	Hom. MR	Seg. MS + MR
504	Hom. MR	Hom. MR	Hom. MR	Seg. MS + R
859	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R

[a] S = (3 + and 4 reactions)
MS = (3 – and 3 reactions)
MR = (2 + and 3 reactions)
R = (1 – and 2 reactions)

be observed that there is a close reciprocal relation between the reactions of all but one of the strains to forms 21 and 14. No such relation exists between the reactions to forms 36 and 14. The data with respect to form 17 are not conclusive. Strains numbers 105 to 349 were read before the rust had developed sufficiently to give accurate results. On the remainder, numbers 374 to 859, the results agree quite well with those obtained for form 21. There seem sufficient data to show that in H–44–24 either the same factors govern the reaction to forms 21, 14, and 17, or if these factors are different they are closely linked. The factors for resistance to form 36 are distinct from those governing resistance to the other group of forms. One of the most interesting observations in this connection is the reciprocal relationship between the inheritance of resistance to forms 21 and 14. The Marquis factor or factors for high resistance to form 14 are allelomorphic

to H–44–24 factors for moderate resistance to form 21. In Marquis also the same factors give complete susceptibility to at least two other forms. The factor relationships in the two parents for rust reaction to forms 9, 14, 15, 17, 21, and 34, may easily be as indicated in diagram 2.

DIAGRAM 2. *Hypothetical factor relationships in H–44–24 and Marquis for resistance to physiologic forms 9, 14, 15, 17, 21, and 34.*

If diagram 2 gives a true indication of the relation, it is impossible in this cross to combine the moderate resistance of H–44–24 to form 21 with the high resistance of Marquis to form 14.

It is evident from these results that the building up of resistance by hybridization must be carried out with extreme care in the matter of selection of parents and testing of the progeny to different physiologic forms. The first essential is a thorough genetic analysis of various parents with respect to the factors controlling resistance to different groups of forms. Not until these data have been obtained can a systematic program be outlined to build up resistance to a large number of forms in one variety.

FIELD REACTION OF F_3 FAMILIES

In the summer of 1927 the hybrid nursery was located at Morden, Manitoba, on the plots of the Dominion Experimental Station. The season was very late. Sowing was not completed until June 15. The natural epidemic was very heavy and all of the physiologic forms being carried in the greenhouse were transferred in pots to the field. The result was a very heavy epidemic, which practically destroyed all of the very susceptible varieties and strains.

The 1054 F_3 lines which had been tested the previous winter to forms 36 and 21 were all grown in the field. The parents were scattered uniformly throughout this nursery and, while Marquis was very heavily rusted and produced only very shrunken grain, H–44–24 was almost entirely free and developed normally with the exception of damage caused by foot rots. It did not appear to be more susceptible to such diseases, however, than other standard varieties.

About September 10 data were taken on the reaction of the F_3 lines to rust. They were classified while standing in the field as homozygous resistant, segregating, and homozygous susceptible. The segregation as

illustrated in figure 7 was so clear cut that this classification was easily made. The uniformly susceptible lines were very obvious. The results of this study are given in table 10.

If field resistance is governed by a single factor pair, the three classes should be obtained in a 1:2:1 ratio. The goodness of fit of the numbers obtained to the theoretical 1:2:1 is given in table 11.

This is not a satisfactory fit, owing to a deficiency in the resistant class which is added to the segregating class. There is a possibility that natural

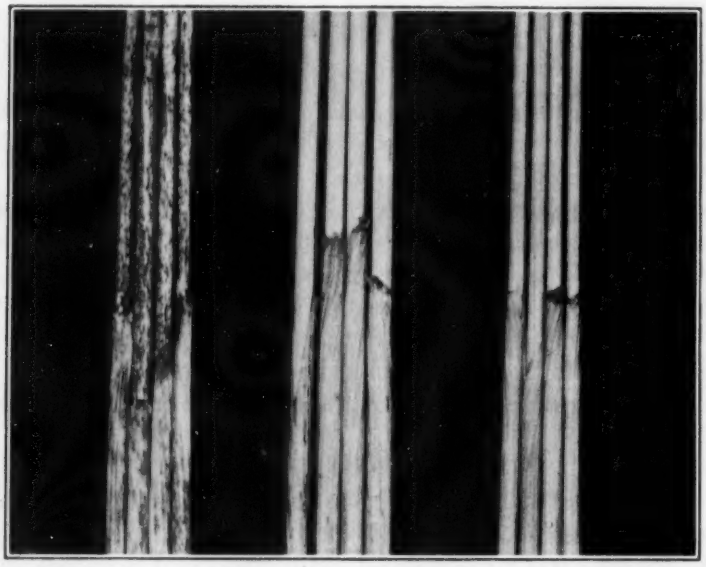

FIG. 7. Field reactions in 1927 hybrid nursery. Marquis, left; H–44–24, right; and a resistant F_3 line, centre.

crossing in the previous generation may have helped to produce such a result. Adding the resistant and segregating classes together, we get 796:249, which is a very good fit to a 3:1 ratio.

RELATION BETWEEN FIELD AND GREENHOUSE RESISTANCE

Any relation between field and greenhouse resistance should be most evident from a study of the families homozygous according to the greenhouse test for resistance or susceptibility to both of the rust forms used. There were 36 of such families susceptible in the seedling stage and 32 resistant. The segregation of these families for rust resistance in the field was studied in detail and the results are as given in table 12. It is evident

TABLE 10.—*Number of F_3 families homozygous resistant, segregating, and homozygous susceptible, as determined by the field reaction*

F_1 plant number	Number of F_3 families			Total
	Resistant	Segregating	Susceptible	
550	17	32	9	58
538	20	33	15	68
535	13	35	16	64
534	8	24	15	47
543	9	29	11	49
541	17	27	17	61
537	18	28	14	60
551	17	52	20	89
548	14	46	12	72
533	3	29	15	47
542	15	27	13	55
545	13	43	13	69
549	3	18	8	29
546	10	30	18	58
539	17	27	13	57
547	9	26	8	43
540	7	38	12	57
544	16	26	20	62
Totals	226	570	249	1045

that in both groups there is a 1:2:1 ratio of resistant, segregating, and susceptible families showing complete independence of the greenhouse test. The results are best summarized in the form of a 2-by-3-fold correlation surface, table 13, for which the calculated value of X^2 is 0.275. For $n=2$ (number of degrees of freedom) P is 0.90 to 0.80 indicating complete independence of the two distributions.

Irrespective of this result it cannot be definitely stated that there is not an observable relation between the two types of resistance. There is a

TABLE 11.—*Total number of F_3 families classified for field resistance as resistant, segregating, and susceptible, and calculation of the goodness of fit to a 1:2:1 ratio*

Actual numbers	Theor. numbers	$(A-T)^2/T$
226	261.25	4.756
570	522.50	4.318
249	261.25	.574
1045	1045	$9.648 = X^2$ P = less than .01, Fisher (2)

distinct difference in the type of segregation in the two groups as shown in table 12. Families in the seedling-susceptible group segregate in the field approximately in a 1:2:1 ratio of resistant, semi-resistant, and sus-

TABLE 12.—*Segregation in the field for rust reaction of two groups of F_3 families*

Homozygous susceptible in seedling stage to forms 21 and 36				Homozygous resistant in seedling stage to forms 21 and 36			
Family no.	Resistant	Semi-resistant	Susceptible	Family no.	Resistant	Semi-resistant	Susceptible
100	All			129	All		
152	do			395	do		
183	do			733	do		
214	do			888	do		
227	do			896	do		
340	do			911	do		
459	do						
883	do						
75	0	40	17	24	27	10	14
256	15	18	10	191	27	1	6
292	2	18	7	278	12	2	10
295	9	23	6	348	14	22	11
419	0	38	17	467	28	6	10
421	3	22	5	560	35	0	15
423	12	41	17	631	47	0	12
470	6	19	8	666	79	2	29
471	20	46	19	670	52	0	20
473	5	23	12	706	29	0	7
615	17	34	13	718	33	0	17
619	10	24	19	719	39	0	9
829	19	35	28	782	64	1	9
836	22	31	18	988	34	8	11
841	14	14	7	192	Segregating nos. small		
854	14	15	13	283		do	
913	29	29	20	285		do	
984	4	30	17	502		do	
104			All	7			All
155			do	210			do
161			do	302			do
164			do	465			do
233			do	580			do
311			do	595			do
347			do	893			do
621			do	944			do
623			do				
748			do				

ceptible plants, while in the seedling-resistant group semi-resistant plants are almost completely absent. An explanation of this is not possible until a more detailed study has been made of the nature of resistance exhibited only by plants approaching maturity. If such resistance is purely morphological the results obtained from the segregation of these families are probably to be expected. It will be remembered that the SR class contains plants practically free from rust but usually with a few pustules on each plant just above the nodes, and in families susceptible in the seedling stage and segregating for resistance in the field the SR plants would be those heterozygous for the factor for morphological resistance which is not completely dominant. In families resistant in the seedling stage to at least two predominating forms, the chances of the development of rust on only a small portion of the plant surface would be much less, and the SR

TABLE 13.—*Two by three fold correlation tables summarizing results from table 12*

Field reaction / Seedling reaction	Homozygous resistant	Segregating	Homozygous susceptible	Totals
Resistant to forms 36, 21	8	18	10	36
Susceptible to forms 36, 21	6	18	8	32
Totals	14	36	18	68

$X^2 = 0.275$. $P = .90$ to $.80$.

class would be accordingly much smaller. In a plant showing only morphological resistance, as described by Hursh (6), there would seem to be greater chances of infection just above the nodes than on any other part of the plant, owing to the fact that the bundles coming out of the node have at that point not extended to the epidermis of the stem.

The exact nature of the resistance here referred to as field resistance is very important. Such families as numbers 100 to 883 in the seedling-susceptible group and numbers 129 to 911 in the seedling-resistant group are indistinguishable from the standpoint of resistance in the field. They are almost entirely free from rust, and if the type of resistance that they show is morphologic they would be expected to show this resistance irrespective of physiologic forms. It is a very pronounced type of resistance and, since it appears to be governed by only a single pair of factors, is extremely important from the plant-breeding standpoint.

GENETIC STUDIES ON OTHER CHARACTERS

Awning

Figure 8 illustrates the spike characters of H–44–24 and Marquis. In F_1 the type was almost intermediate but resembled Marquis more closely than the H–44–24 parent. In F_2 the ratio of awnless and intermediate to awned was 791 : 267. The deviation from a perfect 3 : 1 ratio is only 2.5 while the probable error is 9.5. This is a very close fit, and the classification of the F_2 plants was checked by the segregation in F_3 families.

FIG. 8.—Spikes of Marquis (left), H–44–24 (right), and F_1 plant (centre).

Persistence of Glumes

The F_2 plants were threshed with a machine made especially for single plant work. It consists of a drum covered with corrugated rubber which operates against a belt of corrugated rubber extending over about five inches of the circumference of the drum. The belt may be adjusted for tightness against the drum. The heads of a single wheat plant of the ordinary free threshing type are threshed in a few seconds by passing them between the rotating drum and the rubber belt. In threshing the plants in this cross it was observed that there was a fairly clear-cut differentiation between the free threshing Marquis type and the type with persistent glumes resembling H–44–24. The former were threshed by passing them

through the machine once while the others required passing through two to four times. Records were kept, therefore, on the remainder of the plants and a ratio of 540 persistent- to 154 free-threshing plants was obtained. Fitting this to a theoretical 3:1 ratio we have a deviation from the theoretical of 19.5 and a probable error of 7.69. The deviation divided by the probable error is 2.54, indicating that the departure from a 3:1 ratio is not particularly significant.

Pigmentation of Seedlings[3]

The seedlings grown in the greenhouse for tests of resistance to rust were examined for coloration when about one inch above the ground. A fairly definite segregation was observed and records were kept of the number of families breeding true for color, segregating, and breeding true for absence of color. The ratio obtained was 413:550:54. On the basis of a differentiation between the parents for this character of two pairs of factors the ratio to be expected would be as follows—

7 breed true for color
8 segregate
1 breeds true for absence of color

The goodness of fit of the actual to this theoretical ratio is shown in table 14.

TABLE 14.—*Number of F_3 families breeding true for color, segregating, and breeding true for absence of color, and goodness of fit to a theoretical 7:8:1 ratio*

Groups	Actual	Theor. 7:8:1	$(A-T)^2/T$
Colored	413	444.9	2.29
Segregating	550	508.5	3.39
Green	54	63.6	1.45
Total	1017	1017	$7.13 = X^2$ $P = .028$

Since P is less than 0.05 the deviation is quite significant. Table 14 shows that the big discrepancy is in the first two classes, the segregating class being much too large. The only class that was perfectly distinct was the homozygous green class. In the uniform pigmented classes a considerable variation due to environmental influences seemed evident so that a number of these may have been classed as segregating. The ratio of homozygous green families to the remainder is 54:963. The theoretical

[3] The coleoptiles of H–44–24 seedlings are of a distinct purplish red color.

1 : 15 ratio is 63.6 : 953.4. The fit here is good since the deviation from the theoretical is only 9.6 and the probable error is 5.21. It seems safe to conclude that the pigmentation of the seedlings is governed by two pairs of duplicate factors.

SUMMARY OF FACTOR RELATIONSHIPS

Putting aside for the present the possibility of linkage between any of the factors concerned, table 15 is a fairly accurate statement of the factor relations in the cross studied.

TABLE 15.—*A summary of factor relationships in the cross, H–44–24 × Marquis*

H–44–24		Marquis	
Factor	Character	Factor	Character
R_m	Resistance of mature plants to stem rust	r_m	Susceptibility of mature plants to stem rust
R_1R_2	Resistance to form 36	r_1r_2	Susceptibility to form 36
s_1s_2	do 21	S_1S_2	do 21
a	Presence of full awns	A	Absence of full awns
G	Persistence of glumes	g	Free threshing
P_1P_2	Pigmentation of seedlings	p_1p_2	Green seedlings

$R_mR_mR_1R_1R_2R_2s_1s_1s_2s_2aaGGP_1P_1P_2P_2 \times r_mr_mr_1r_1r_2r_2S_1S_1S_2S_2AAggp_1p_1p_2p_2$

THE INHERITANCE OF MATURE PLANT RUST RESISTANCE IN RELATION TO MORPHOLOGICAL AND OTHER CHARACTERS

As previously pointed out, the parent H–44–24 is a vulgare wheat derived from an emmer × Marquis cross. It carries with it in addition to rust resistance other characters derived from the emmer parent. It is bearded, has very persistent glumes, and the coleoptile when just emerging and until the plant is several days old is distinctly of a purplish red color. In these three characters it is sharply contrasted with Marquis. If only a few entire emmer chromosomes were transferred to H–44–24 there would seem to be considerable likelihood of an observable linkage between some of these characters. The segregation for these characters was therefore studied in some detail and an attempt made to detect any tendency for a pair of characters to be inherited together.

The method of measuring such relationships is shown in tables 16 to 30. These are contingency tables in each of which a pair of characters are considered. A direct measure of the relationship between the distribution for the two characters may be obtained by calculating X^2 and determining the value of P from Pearson's tables (9). In determining P from the tables,

Fisher's (2) suggestion has been followed relative to the number of degrees of freedom. Thus in a 3-by-3-fold table there are four degrees of freedom, and one enters Pearson's tables under n = 5. A summary of the values of P is given in table 31. In interpreting these results it is safe to assume that, if the value of P for any given distribution is higher than 0.05, there is no significant evidence of correlated inheritance. A P of 0.05 means that any association that exists between the two distributions might occur by chance in one out of twenty cases.

TABLE 16.—*Distribution of F_3 families for awning and field rust reaction*

Field rust reaction	Awned	Awning Segregating	Awnless	Totals
Res.	57	117	51	225
Seg.	149	291	127	567
Susc.	57	118	70	245
Totals	263	526	248	1037

$X^2 = 3.97$ $P = 0.411$

TABLE 17.—*Distribution of F_3 families for awning and seedling reaction to form 36*

Seedling reaction to form 36	Awned	Awning[a] Segregating	Awnless	Totals
Susc.	13	13	23	49
Seg.	130	213	203	546
Res.	35	67	46	148
Totals	178	293	272	743

$X^2 = 6.24$ $P = 0.184$

[a] Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 18.—*Distribution of F_3 families for awning and seedling reaction to form 21*

Seedling reaction to form 21	Awned	Awning[a] Segregating	Awnless	Totals
Susc.	67	100	96	263
Seg.	116	186	138	440
Res.	18	34	27	79
Totals	201	320	261	782

$X^2 = 2.49$ $P = 0.649$

[a] Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 19.—*Distribution of F_2 plants for awning and persistence of glumes*

Persistence of glumes	Awned	Awning Intermediate	Awnless	Totals
Per.	42	60	52	154
Int.	98	133	112	343
Free	42	90	65	197
Totals	182	283	229	694

$X^2 = 4.26$ $P = 0.375$

TABLE 20.—*Distribution of F_3 families for awning and cotyledon color*

Cotyledon color	Awned	Awning[a] Segregating	Awnless	Totals
Pig.	98	131	138	367
Seg.	128	195	175	498
Green	9	26	19	54
Totals	235	352	332	919

$X^2 = 4.34$ $P = 0.366$

[a] Data taken on F_2 plants only. The class referred to here as segregating is represented by intermediate-type F_2 plants.

TABLE 21.—*Distribution of F_3 families for seedling reaction to forms 21 and 36*

Seedling reaction to form 21	Seedling reaction to form 36			Totals
	Susceptible	Segregating	Resistant	
Susc.	14	113	41	168
Seg.	15	206	53	274
Res.	1	40	18	59
Totals	30	359	112	501

$X^2 = 7.69$ $P = 0.106$

TABLE 22.—*Distribution of F_2 families for persistance of glumes and seedling reaction to form 36*

Seedling reaction to form 36	Persistence of glumes[a]			
	Persistent	Segregating	Free	Totals
Susc.	7	11	9	27
Seg.	57	144	92	293
Res.	15	40	26	81
Totals	79	195	127	401

$X^2 = 1.01$ $P = 0.908$

[a] Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 23.—*Distribution of F_3 families for cotyledon color and seedling reaction to form 36*

Seedling reaction to form 36	Cotyledon color			
	Pigmented	Segregating	Green	Totals
Susc.	21	27	1	49
Seg.	219	294	35	548
Res.	59	79	10	148
Totals	299	400	46	745

$X^2 = 1.59$ $P = 0.807$

TABLE 24.—*Distribution of F_3 families for persistence of glumes and cotyledon color*

Cotyledon color	Persistence of glumes[a]			
	Persistent	Segregating	Free	Totals
Pig.	52	122	61	235
Seg.	60	146	90	296
Green	6	14	14	34
Totals	118	282	165	565

$X^2 = 3.82$ $P = 0.433$

[a] Data taken on F_2 plants only. The class referred to here as segregating is represented by intermediate-type F_2 plants.

TABLE 25.—*Distribution of F_3 families for seedling reaction to form 36 and field rust reaction*

Field rust reaction	Seedling reaction to form 36			
	Resistant	Segregating	Susceptible	Totals
Res.	51	157	12	220
Seg.	131	395	35	561
Susc.	58	162	22	242
Totals	240	714	69	1023

$X^2 = 3.16$ $P = 0.534$

TABLE 26.—*Distribution of F_3 families for seedling reaction to form 21, and field rust reaction*

Field rust reaction	Seedling reaction to form 21			
	Resistant	Segregating	Susceptible	Totals
Res.	25	105	64	194
Seg.	47	284	190	521
Susc.	21	120	77	218
Totals	93	509	331	933

$X^2 = 2.65$ $P = 0.620$

TABLE 27.—*Distribution of F_2 families for persistence of glumes and field rust reaction*

Field rust reaction	Persistence of glumes[a]			
	Resistant	Segregating	Free	Totals
Res.	35	72	35	142
Seg.	86	192	112	390
Susc.	34	81	51	166
Totals	155	345	198	698

$X^2 = 1.69$ $P = 0.790$

[a] Data on persistence of glumes were taken on F_2 plants. The class here referred to as segregating came from intermediate plants.

TABLE 28.—*Distribution of F_3 families for cotyledon color and field rust reaction*

Field rust reaction	Cotyledon color: Pigmented	Segregating	Green	Totals
Res.	87	126	11	224
Seg.	228	310	31	569
Susc.	101	140	7	248
Totals	416	576	49	1041

$X^2 = 2.82$ $P = 0.590$

TABLE 29.—*Distribution of F_3 families for persistence of glumes and reaction of seedlings to form 21*

Seedling reaction to form 21	Persistence of glumes[a]: Persistent	Segregating	Free	Totals
Susc.	49	96	52	197
Seg.	66	188	103	357
Res.	17	20	16	53
Totals	132	304	171	607

$X^2 = 7.73$ $P = 0.104$

[a] Data taken on F_2 plants only. The class referred to here as segregating is represented by intermediate F_2 plants.

TABLE 30.—*Distribution of F_3 families for cotyledon color and seedling reaction to form 21*

Seedling reaction to form 21	Cotyledon color: Pigmented	Segregating	Green	Totals
Susc.	105	144	14	263
Seg.	172	248	19	439
Res.	33	43	3	79
Totals	310	435	36	781

$X^2 = 0.71$ $P = 0.936$

TABLE 31.—*Summary of values of X^2 and P from tables 16 to 30*

Table	Characters[a]	X^2	P	Table	Characters	X^2	P
16	AM	3.97	0.411	25	MR_1	3.16	0.534
17	AR_1	6.24	0.184	26	MR_2	2.65	0.620
18	AR_2	2.49	0.649	27	MG	1.69	0.790
19	AG	4.26	0.375	28	MP	2.82	0.590
20	AP	4.34	0.366	29	R_2G	7.73	0.104
21	R_1R_2	7.69	0.106	30	R_2P	0.71	0.936
22	R_1G	1.01	0.908				
23	R_1P	1.59	0.807				
24	GP	3.82	0.433				

[a] Explanations of symbols:

A—awning
G—persistence of glumes
P—Cotyledon color
R_1—seedling resistance to form 36
R_2—seedling resistance to form 21
M—mature plant resistance

Linkages are of course very difficult to detect when one or both of the characters are governed by two pairs of factors, but with regard to the characters represented by A, M, and G the results would appear to be quite conclusive.

DISCUSSION

The existence in such a variety as H–44–24 of a type of rust resistance which does not become evident until the plants are about half way to maturity is of particular significance from the standpoint of breeding work. It appears to be a very pronounced type of resistance amounting almost to immunity and, while there is no definite evidence to the effect that it reacts in the same manner to all physiologic forms, there seems good reason to believe that it does react in the same manner to all of the forms commonly found in the Northwest. From the breeding standpoint, therefore, this entire group of forms may perhaps be considered as an entity. This considerably simplifies the problem especially since we seem to have fairly good evidence that the type of resistance referred to is governed by only a single pair of factors.

Whatever may be the practical value of the results reported here, it is evident that in all breeding problems studies of the inheritance of rust resistance should take into consideration both the seedling reactions and the mature plant reactions of the parents to different physiologic forms. If a mature-plant type of resistance exists in one or both of the parents, it is obvious that a close agreement between seedling and field results is not to be expected.

SUMMARY

1. A cross was made between H–44–24, a vulgare derivative from a Marquis × emmer cross, and Marquis.

2. The H–44–24 parent is moderately resistant in the seedling stage to several forms of *Puccinia graminis tritici*, including forms 14 and 21, and highly resistant to form 36. Marquis is susceptible to a large number of forms, including forms 36 and 21, but is highly resistant to form 14.

3. The high resistance of H–44–24 to form 36 is dominant in the F_1 generation but its moderate resistance to form 21 is recessive. Tests on 1000 F_3 families clearly indicated that there is a difference between the parents of two pairs of factors for resistance to form 36, and the same is probably true for resistance to form 21.

4. Studies of the reactions of F_4 lines breeding true for reactions to forms 21 and 36 showed that all of the lines possessing moderate resistance to form 21 gave the same reaction as Marquis to this group of other forms. This group of forms contains form 14 to which Marquis is highly resistant and others such as 21 and 17 to which it is highly susceptible. It would seem that in this case the same factors govern resistance to one form and susceptibility to another.

5. First generation plants susceptible to form 21 in the seedling stage showed very high resistance to the same form as they approached maturity. Studies of the field reaction of one group of F_3 lines breeding true for susceptibility in the seedling stage to forms 21 and 36 and of another group breeding true for resistance to forms 21 and 36 showed that segregation in the field was quite independent. The field results indicated that resistance in the field is controlled by a single pair of factors only.

6. An attempt was made to detect any tendency towards linkage in the inheritance of mature plant resistance, seedling resistance to form 21, seedling resistance to form 36, awning, persistence of the glumes, and cotyledon color. No such tendency was observed.

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PROGRESS OF RUST STUDIES[1]

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I count it one of the rare and notable events of my life that I am privileged to meet with fellow scientists in this great northern city. I have always thought of Winnipeg as in the far north, just as I have heard people in New England speak of Indiana as in the far west; and yet I know full well that this is just the edge of a great country of unlimited possibilities that stretches far to the northward, both on the right hand and on the left hand. As I gazed out of the window of the incoming train after crossing the international boundary, I saw no foreign scenery, but just the familiar stretch over rolling prairies that recalled my childhood days in Iowa. So I find Winnipeg in its outward setting very homelike, and no less so in the cordial and sincere greeting I have received. However, it was not only the charm and bustle of a great city and the exhilaration of an expanding territory that induced me to make a thousand-mile trip in winter time in defiance of Boreas, but also the desire to secure first hand information regarding the young, but already famous, Dominion Rust Research Laboratory, the only one of its kind in the world, which is making such notable contributions to the increasingly important problems of the science, and, in addition, to greet my fellow workers whose signatures are familiar but whose faces and voices I do not know. I am grateful for the opportunity you have afforded for this visit, and in return I know you expect me to say something about the rusts.

Of course you and I know what we mean by the term "rusts," but sometimes for the benefit of others we say rust-fungi or plant-rusts and thus screen out all thought of rusts of metals. Yet there are rusts of plants that do not belong in the group of rusts with which we deal, for instance, rust of strawberry and many others. To be still more exact we introduce the term "Uredinales" into titles of articles. This is a comparatively new word, and there is no question what it covers if one happens to know the usage. But it is founded upon the genus *Uredo*, which for a time took in all kinds of fungi, and even today does so to some extent, and is treated among the rusts only as a form-genus. The word *Uredo* comes from the Latin verb *urere*, to burn or blast as with fire or a scorching wind, and is more suitably applied to the smuts than to the rusts.

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In early Grecian times, when Aristotle and Theophrastus wrote, *erysibe* was the term designating the rusts, but it was diverted by Linnaeus and others to the surface mildews. The corresponding word in Latin was *robigo*, which meant just what we mean today, popularly and scientifically, by "rust." But this word also fared badly at the hands of the early scientists. Some years ago I spent considerable effort in ascertaining whether *Robigo* could not be re-established as a good genus of rusts, but all in vain. If I had succeeded, we might have had the pleasing name *Robigales* for the order, a name with a clean historical record. Now when I am asked my special line of study I answer, "The rusts," which I fancy usually conveys about the same exactness of meaning as my reply when I was asked in Germany in *ante bellum* days from what country I came. I proudly answered, "From America—I am an American." But I was humbled by what followed. "Oh, then you probably know some of my relatives in Rio de Janeiro." And I was forced to explain that I had meant only the United States. How could I have said I was a Unitedstatesian?

Before I take up some of the problems of the rusts as they present themselves today, let me outline a few historical episodes to bring to mind the slow unfolding of rust knowledge. It was on the twenty-fifth day of April about the beginning of the Christian era, that the poet Ovid early one morning was strolling along the Claudian way not far out from Rome. This was the time of year when the advent of the wheat rust was especially feared. Down the road came a grave procession of husbandmen and their dependents, headed by a group of white-robed priests. The procession turned into a grove and gathered about an altar; and Ovid, after the manner of writers always alert for material for another article, mingled with the worshippers and recorded the proceedings.

This solemn festival, the Robigalia, which had been held annually for many centuries, was in honor of the god Robigo, who controlled the rust. After the sacred flame was placed on the altar a lengthy prayer was offered, asking the protection of the god that the fields might be free from rust. It was not unlike in phrasecology and purport the prayers offered by devout Christians of the present day when drought or some other calamity threatens disaster. After the prayer, in accordance with the customs of the time, sacrifices were offered of wine, grain, and young animals.

What more could have been done? The knowledge of rusts had not advanced materially since prehistoric times. This kind of injury to the crops was popularly supposed to have had its origin in some revengeful act. The best remedies known, aside from prayer and sacrifice, were the sensible advice to avoid wet and low lands, combined with such fantastic methods as sticking sprigs of laurel about the fields and burning piles of straw.

Such ignorance of the nature and treatment of rust changed but little during the next seventeen centuries. In England in 1602, Shakspeare in his play "King Lear" said "a foul fiend" scattered distress among men, beasts, and inanimate objects, including the rust on wheat. A better understanding of the rusts first came when the microscope began to be used during the latter half of the seventeenth century, crude as the instrument was in its early form.

The second episode I wish to present occurred in Florence, a city that encouraged arts and science, greatly antedating the culture of Rome. It was in December, 1729, that a scrupulously dressed man of patrician bearing, well-known as Señor Micheli, passed along the streets with a small quarto in full calf binding under his arm. It contained about two hundred pages and half as many steel engraved plates of plants, mostly of the lower orders, each plate dedicated to a distinguished personage. This volume contained the description and a full page illustration showing gross and microscopic details of the first recognized genus of rusts, that of *Puccinia*, named in honor of Thomas Puccini, a physician in Florence and lecturer on philosophy and anatomy at the Academy.

When you are in Florence go to the Uffizi palace and inspect the statues surrounding the quadrangle. This is the hall of fame for the city of Florence. Near the end of the eastern portico toward the river will be found the statue of Peter Antonio Micheli, the only botanist in this assemblage of eminent personages. It is a dignified, courtly figure, with a sprig of jasmine in the left hand, another sprig together with a boletus and an agaric dropped at his feet, and the right hand supporting the precious quarto of new genera. This volume inaugurated the taxonomic study of the rusts.

As a separate group of fungi, however, the rusts were first recognized about 65 years later when Persoon added the genera *Uredo* and *Aecidium* to that of *Puccinia* with a number of species under each genus. Still later he gave the name Uredineae to the group. In the meantime this orderly taxonomic progress received an upheaval when the younger Hedwig, in an ill-fated study sponsored by A. P. DeCandolle, proposed the genus *Gymnosporangium* to replace Micheli's genus *Puccinia*. The most strenuous and persistent efforts at various times to undo this injury to uredinology have been unsuccessful. The study of the rusts at the beginning of the nineteenth century was now fully launched, and both taxonomy and morphology attracted many able scholars.

The third episode which I have chosen to illustrate the unfolding of the knowledge of rusts is laid in Berlin, and introduces a young man of 22 years of age who had just graduated from the University. It was April,

1853, and from the press had come a treatise of 144 pages on the blight fungi, meaning the smuts and rusts and the diseases caused by them. It would have done credit to a mature mind, but as the work of a boy in his undergraduate days it indicated genius. Besides recording important morphological results, the author traced the course of the mycelium in the tissues of the host and detected the origin of the sori. He argued that, as such fungi were capable of reproduction by spores, they were independent organisms, and not, as most people thought, an excrescence or exudation of morbid tissues. Being independent organisms, they must cause the disease with which they are associated and not be its product, as generally held. Neither did he believe that the teliospores were parasitic upon the uredinia although found in the same sorus.

Thus Anton de Bary settled for all time some of the basic problems of the rusts, although he still maintained the old notions that forms belonging to the genera *Aecidium*, *Uredo*, and *Puccinia* were distinct species, and that it was impossible for barberry rust to turn into wheat rust. But he did not allow his inherited ideas to interfere with his studies, and in his second paper ten years later he was able to clear up these and many other erroneous beliefs. He introduced the culture method of study. He found that the rusts have a succession of spore-forms, and that *Aecidium* and *Uredo* are only form-genera. He reformed the terminology by using aecidiospore, uredospore, and teleutospore. Most important of all, he detected and demonstrated an alternation of hosts, for which he supplied the term heteroecism.

It is difficult to realize that the dawn of a recognition of the rusts as individual objects dates back scarcely two hundred years, before which time there were only superstitions and wild guesses as to their nature, and that a fairly comprehensive idea of the rusts covers little more than a quarter of this period, in fact only about sixty years. The shortness of this latter period was impressed upon me forcibly by a personal incident. While on a brief visit to Strassburg in 1888 I met Professor de Bary, then apparently in the prime of life, although it was only a few months before his untimely death. My own active participation in solving rust problems, therefore, overlaps that of de Bary, and in fact covers more than two-thirds of the whole 60-year period. It should need no argument to convince any one that the unfolding of knowledge regarding a group of organisms so large and complex as the rusts must have gained only fair headway after six decades, and that the opportunities for profitable research are still unlimited.

In selecting topics to represent the general progress of rust studies at the present time it will be well to begin at the beginning. And what is the

beginning in any subject in natural history? Clearly it is the recognition of individual kinds and their designation by names, permitting of record and discussion. It is the nomenclature question that is fundamental, for names are the worker's tools. It is a hackneyed subject and generally distasteful, but one that every writer necessarily deals with.

The rules of nomenclature should be the same for rusts as for any other group of plants. The methods of applying names and establishing their validity constitute the large part of the so-called rules of nomenclature, and such rules to be generally useful must be formulated and interpreted by an authoritative body, duly appointed and accepted by practically the whole mass of working botanists. Much progress has been made toward such a desirable situation, but we have not arrived, although I believe to do so will be eventually possible. Everyone who applies a name to a new species or desires to use correctly a name already established—if he has the good of the science at heart—must wish to feel that he is proceeding in a manner generally acceptable to his fellow workers. The name is a means of identification, and is not to be confounded with any question of classification.

In this connection I think it is pertinent to ask, what is a name, and how is it to be used to serve its purpose? Judging from current writings there must be much diversity of opinion. Is it the generic or the specific part that carries the essential feature of identification, or is it the combination? It is in fact all three, but in varying degrees of importance. The only unalterable feature is the specific part, which is presumably the first and oldest designation for the species, or one which for sufficient reasons has been accepted as a substitute. This essential part is validated when combined with some genus name to show in what group it belongs. The specific part in combination with any suitable genus constitutes the name; thus, *Puccinia glumarum, Dicaeoma glumarum,* and *Pleomeris glumarum* are all correct names of a particular species of rust, and the choice lies with the user.

It has become customary, and in some cases is highly serviceable, to append a bibliographical citation to indicate the source of the original application, but such citation is no part of the name. It is a superfluous nicety to write *Puccinia glumarum* (Schmidt) Erikss. and Henn., except when required for the sake of uniformity, as there is but one rust called *Puccinia glumarum.* But if one desires to mention the common barley rust as *Puccinia hordei,* it is well to remember that this name has been applied to two distinct rusts. *P. hordei* Fuckel is the rust on wild barley occurring along the Pacific Coast and in Europe, but *P. hordei* Otth is the rust of the cultivated barley. Fuckel's name is the older one, and so

the common barley must take the next oldest name, *P. anomala*, unless the citation is used.

All this is highly elementary, but may be taken as an indication of progress in the application of Latin names to the rusts. Any serious attempt to establish the status of rust names dates back only a few years, and even now a large amount of work is needed to determine the correct application, especially of the older names. What would be highly serviceable is a comprehensive monographic treatise to be used as an authority, not a compilation like Saccardo's Sylloge, useful as that is, but a treatise more like Sydow's Monographia Uredinearum, with each name properly substantiated. This could be done by countries or large areas, and need not await a universal study. In the meantime sufficient progress has been made in providing authoritative treatises to permit omission in most instances of the obnoxious personal advertisement, as interpreted by C. G. Lloyd, except in formal taxonomic articles. At any rate it should be borne in mind that such an appendage is not a part of the name, and is only required for bibliographical identification.

This leads naturally to the question of new species. When I began my work with the rusts there was a certain amount of opprobrium attached to the publication of new species. The work of Mr. Peck and Mr. Ellis was rated as rubbish before the wheels of botanical progress. It was said that anyone might send a specimen and get a name for it by return mail, implying that species were manufactured for the asking. The taxonomic worker of today knows there was small ground for such accusation. It is to the untiring and conscientious devotion of Mr. Peck and Mr. Ellis and others of like spirit that we of today are enabled to have sufficient working material with which to untangle and arrange the hundreds of genuine species and their unlimited variations. In spite of the multiplicity of so-called species, both good and bad, there are still many true species and forms that await detection, and one need not go to the tropics to find them, as witness the admirable results achieved by Messrs. Fraser and Faull in Eastern and Western Canada.

The identification of species is not so simple a problem as it was twenty-five or thirty years ago. It is now realized that to describe adequately a species for purposes of full recognition it is necessary to take note of its several states or metamorphoses, and not only to describe each but show which, if any, are absent. Even then the task is not complete, for there are the hosts to be identified. But there are two phases in this situation: one relates to the application of the name, and the other to the question of classification.

It is manifestly impossible in many instances for the person who desires to describe a new species to have all the possible stages in the life-cycle at

his command. The more or less partial description must be drawn from the specimen in hand, and to that material the name is attached. It can be left to the future and to others to supply the missing items and to decide if the name has been antedated, or if the same or some other name has been applied to another stage of the same species. The *sine qua non* is an accurate description of a single definite object. To discourage the description and naming of forms thought at the time to be distinct species would be to lose to science much valuable data.

When it comes to the problem of classification, however, the subject of species and their names assumes a wholly different aspect. Let us illustrate with a comparatively simple example. *Puccinia andropogonis*, a common grass rust on the various species of *Andropogon* throughout North America, is known to develop its aecia on *Pentstemon* and *Comandra*, making two races, differing slightly in morphological characters. It is also probable, although not yet positively proved by cultures, that *Aecidium onobrichydis* on various leguminous hosts, and *A. polygalinum* on *Polygala senega*, bear the same relationship. There is not much doubt that other aecial forms which now bear independent names are also to be included. In the happy days of not very long ago all these forms could be treated as so many species; now, however, we are disposed to put them under one name. But our task with this one species is not completed when all the aecia have been rounded up and impounded. There are a number of correlated short-cycle rusts on the aecial hosts that undoubtedly belong in this group and have the same ancestry, such as *Puccinia pentstemonis, P. seymeriae, P. comandrae*, and possibly others. Now are all these forms, these so-called species, to go under one name, and constitute the variables of the true *Puccinia andropogonis?* That seems to be the conclusion according to our present tendency.

When such a diversified species—and there are many of them—is fitted into a natural classification, and we look for clues to relationship with other species, are we to seek them in the gametophytic or the sporophytic phase of the species? Some persons consider the teliosporic state of a species as the most important. But to do so one must omit from consideration the question of gametophyte and sporophyte and the influence of the host, for the telia of the long-cycle and of the short-cycle forms together embrace both sporophyte and gametophyte and the whole gamut of phanerogamic possibilities. If we select the sporophytic phase as the more important for indicating relationship, then only the telia of the long-cycle forms and their hosts are in evidence. But some botanists consider the gametophytic phase as the older and more primitive generation; they believe that it presumably antedates the sporophyte in evolution, and should therefore more definitely

indicate the course of phylogenetic evolution and the degree of relationship. This would make the aecia and their hosts, which are also the hosts of the correlated short-cycle forms, more important in tracing relationship and deciding position in the scheme of classification. Herein lies a puzzle no one has yet solved, a problem that lies at the very threshold of taxonomy for the Uredinales. This problem is the chief reason why our classification of the rusts is yet artificial and chaotic. In other groups of plants, morphological characters largely determine relationship; but in the rusts they are only of coördinate importance, and the bearing of the host must always be considered.

The most notable advance in rust studies during the last few years has been along the line of physiological specialization and breeding for resistance. This is a fascinating field for research, and fortunately has attracted many able investigators. The assiduity with which it is pursued springs from the same motive that fixed the attention of the ancient Greeks and Romans and of students all along the modern pathway from de Bary to Stakman—its economic importance. In the attempt to solve the very difficult problem of preventing financial loss from rusts, the science of uredinology has greatly benefitted.

The chief attack along this line has been made on stem rust, and the prospect is bright for a practical control of this greatest source of injury to crop production. It may not be possible to banish rust from our grain fields in the same degree that yellow fever has been wiped out in most regions where it once flourished, but it should be possible to restrict it to a negligible amount. In the meantime, while that desirable end is being attained, a vast amount of contributory information about the nature and habits of the rusts in general is being unearthed.

In the various lines of inquiry nothing more striking has come to light than the marvelous degree of specialization found in some of the cereal rusts. In the stem rust, *Puccinia graminis*, not only are there now recognized half a dozen or more varieties—or races as they are commonly called by mycologists—according to the genera or species of hosts harboring them, but each race may be subdivided into many physiological forms. In stem rust of wheat such recognized forms run into the scores. They are too numerous and too shadowy for names, and so are given numbers. Of course these numbered forms can be subdivided in some cases. The possible computation is startling. But if this can be said of stem rust, the mathematical possibilities of leaf rust mount into the empyrean, and it would seem to be a task for the gods to trace them. This comes from the fact that while stem rust has a single form of aecium on the barberry, leaf rust has many forms on highly diverse and distantly related hosts; conse-

quently the possibilities of specialization extend both to the aecial and telial sides of each race. The study of leaf rust has barely begun, and even the boundary of the species has not yet been determined, a species which promises by its amplitude and ubiquity to justify the early name of *Puccinia rubigo-vera,* the truly representative rust.

While it has been recognized from earliest times that rusts are spread from plant to plant by wind-borne spores, yet a direct demonstration and study of the method was not undertaken until 1881 when Marshall Ward, in India, caught the spores of the coffee-leaf disease on slides placed at varying distances and in different directions from the diseased trees. The use of sticky slides has been greatly extended in recent years by the aid of various kinds of spore traps, and the placing of these has taken into account vertical as well as horizontal distribution, since Stakman and his co-laborers introduced the use of the aeroplane. Probably the most successful and conclusive studies regarding the direction and extent of wind-borne spores are the result of recent stationary and aeroplane observations by the Dominion Rust Research Laboratory. These studies have supplied valuable data, and the long debated question whether the initial infection of grain fields in the north comes from locally overwintered urediniospores or from wind-borne spores from the south is again shifted to the latter view. The problem of the source of spring infection is of great economic importance and becomes more insistent as one proceeds northward; and nowhere can its solution be undertaken with such clear hopes of detailed success as in Manitoba. The solution, however, requires more extended and resourceful methods than at present employed; in fact nothing less than a well-directed and well-equipped army of observers will determine when and from where the winds begin to bring infection, although the question of overwintering of urediniospores could be pursued with less cooperation. As to the wind, it will be necessary to be wiser, at least in the spring time, than the men of old who could "not tell whence it cometh and whither it goeth."

In a recent study of geographical distribution of rusts in the arctic regions it has been plausibly suggested by Lind of Denmark that the winter winds playing over the surface of the snow, are the chief distributing agents. Possibly it may be true in a modified degree of the two most prominent cereal rusts in their northern limits, stem rust and leaf rust of wheat These rusts may possibly develop strains with unusually hardy urediniospores that overwinter and are scattered by the wind. The thought is suggested by the recent discovery, again emanating from a Canadian experiment station, of a strain of stem rust with urediniospores having deeply colored walls and colorless contents. These are characteristics that accompany resting spores,

and in some manner have to do with the capacity of the spore to withstand untoward environment. They are the most prominent features in the transformation of ordinary urediniospores into resting amphispores. But whether there is necessarily any relation between color changes and hardiness has not yet been studied experimentally.

Two other matters are suggested by the finding of deeply colored urediniospores with colorless contents. What is the chemical nature of the coloring matter of rusts? Very little is known about it, and few attempts at analysis have been made. It is said to be an oily substance. It is usually abundant in the young cells of the mycelium, primordia of the sori, and in the early stage of all sorts of spores, unless exception be made of the pycniospores, the so-called spermogonia. It may be a soluble carotinoid, as tests have indicated. It is usually some shade of yellow. It shows most in the oil globules and other food supplies within the cell, and may itself be a nutrient, and possibly associated with some vitamine. The coloring matter of the cell wall, on the other hand, is rarely yellow, but some shade of orange or reddish brown, and does not respond to the same reagents as that of the cell contents; neither is it available as a nutrient. Whether the basic structure of the color substance both in the cell contents and the wall is the same or not, is still a question of divided opinion awaiting demonstration.

Finding of deeply colored urediniospores with colorless contents was announced as a case of mutation—a color mutation, of course, like finding a red ear of corn or a white-flowered bluebell. If a true mutation, as it seems to be, it is probably to be rated the first authentic instance observed among the rusts, although this method of variation has often been assumed to account for many morphological peculiarities. Probably the most important feature of the discovery lies in the emphasis to be placed upon the study of variation in pedigreed cultures, a field of research scarcely touched, and yet holding the greatest possibilities for an understanding of the origin and permanency of the diversified forms and potentialities among rusts, especially among such highly variable species as the stem and leaf rusts.

There are other ways of attacking the problem of rust variability arising from the single-spore culture method. The brilliant achievement emanating from this laboratory, the demonstration that pycniospores from two strains of sunflower rust or stem rust of wheat when intermingled induce aecia, and when kept separate do not, makes a fair beginning to the broad field of heterothallism and its possibilities. We may sometime know if the rusts have two sexes, or four sexes, or no sexes at all but only an assortment of nuclei or possibly of chromosomes. It may be that the way is open for a broader application of the principles of genetics than heretofore employed

in the solution of rust problems. Some aid is derived from cytology, especially in tracing the course of the nuclei during maturation and germination of the teliospore, as illustrated by the invaluable studies of Dodge. Whether the cytologists can ever utilize the behavior of the chromosomes in the rusts is doubtful, for at present they have not been counted with certainty, and their part in heredity is unknown.

When the problem of heredity is taken up in earnest in its broadest aspect, physiological factors such as susceptibility and immunity of the host, which now are chiefly uppermost because they have to do with economic problems, will take their place with many other features of the subject in which the morphological side will play a large part. It may be we shall find out why, or at least how, the pores in the urediniospores of a species come to be in the upper part of the spore, as in *Uromyces appendiculatus* on cowpeas, while at the equator in the same species on field beans.

In speaking of pores one is led to ask what determines the location of pores. It is easy to see how a pore at the apex of a teliospore that remains attached to the host, as in cereal rusts, is most efficiently placed to bring the germ-tube into the air for the distribution of the basidiospores. But why are the pores of urediniospores numerous and yet never at the apex of the spore, even when the spores are transformed into a resting spore and remain attached to the host? Although both urediniospores and aeciospores usually have more than one, often many pores each, in a few instances urediniospores have only one pore, as in some Carex rusts, and even then it is not at the apex. So far as I know, Dr. Buller is the only person who has made any suggestion regarding the pore situation. He expresses the opinion that with several pores the provision is made so "that at least one pore shall be on the side of the spore which happens to be turned towards the epidermis of the host-plant," and thus assist germination.

Every pore appears to be potentially capable of giving exit to a germ-tube, and often a germ-tube does appear from each pore. Do they all continue to grow and under favorable circumstances form mycelium? One would be led to infer as much from the manner in which observations on germination are usually presented. But a cell to flourish must have a nucleus, or conjugate nuclei as the case may be; and as only one such exists in each normal spore, only the germ-tube which receives it can continue to grow. And what determines the movement of the nucleus? Apparently the mechanical drift of the enclosing cytoplasm. Consequently the germ-tube that grows most rapidly from an aeciospore or urediniospore sucks in the nuclear mechanism along with the forward movement of the protoplasm and is fitted for continued growth, while the others must

perish. So we come around to agree with Dr. Buller that the most favorably placed pore "assists the process of germination" and the continued life of the individual rust.

There is something peculiarly intriguing in the relation of the uredinial generation to the existence of rusts in general. To be sure, it is the chief repeating factor, and in the case of cereal rusts and some others, particularly tropical species, it seems more aggressive and important than any other part of the life-cycle. Yet there are many species, all the short-cycle forms and many long-cycle forms like those of the genus *Gymnosporangium*, that flourish without uredinia. Some of these, for instance the hollyhock rust, are just as intensive and spread as readily as any propagated by urediniospores. Nevertheless the balance of facts that press upon one's attention force the impression that in some way the uredinia are an important factor, even an increasingly important factor, in the existence of rusts as a whole. Of the 60 unattached uredinia listed as species under *Uredo* in the North American Flora, all except four or five are tropical or subtropical forms, indicating the rarity of their telial forms. In temperate regions, as well as in the tropics, many species utilize only the uredinial stage, except under special conditions, for instance, the iris and blue-grass leaf rusts east of the Rocky Mountains; while most of the grass, sedge, and composite rusts show a great preference for uredinia under all climatic conditions.

One is almost led to think that the chief drift in the evolution of the rusts is toward the elimination of other spore-forms and the supremacy of the urediniospore. Such a culmination seems to have been reached in a violet rust of Southern Europe, *Uredo alpestris*, since all attempts to find any other stage have failed. Such an argument can be ably supported by appealing to the well-nigh generally accepted theory at present that the fern rusts are the modern representatives of the most ancient line of rusts. There are six genera of fern rusts known, all heteroecious or presumably so, and all with uredinia as a prominent generation. Moreover, two of the genera, *Urediniopsis* and *Hyalopsora*, have specially modified urediniospores fitting them for resting spores; that is, they possess amphispores in addition to the ordinary form. It is difficult to escape the conviction that in the uredinial generation the rusts are endowed with their strongest means for perpetuation. It is no wonder, then, that the economic necessity of fighting the spread and destructiveness of urediniospores is a battle royal.

On the other hand, however, when a species shows a tendency to shorten the life-cycle, it is the uredinial stage that is first omitted. When conditions for the growth of the rust are poor, as in over-ripened or poorly

nourished tissues, the production of urediniospores is restricted and may even be completely inhibited. This first came to my attention when I cultivated *Cronartium cerebrum* on young oaks in which the annual growth was completed. The aecial infection was followed directly by telia, and either no uredinia or only a few appeared. A similar procedure in other rusts, including those on cereals, has been observed and commented upon by several persons. The same general tendency is manifested when the growing season is shortened, or made less favorable by lower temperature or drought, as in high altitudes or latitudes. The same restrictions to growth may act to eliminate the aecia as well as the uredinia, and then a short-cycle form results. This transformation is observable in such species as *Puccinia podophylli* on the may-apple and *Gymnoconia interstitialis* on blackberries and dewberries. Autoecious species like these, however, in which the change from a long-cycle form without uredinia to a short-cycle form is readily observable, are said to be unstable or mutable. But the process can scarcely be called a mutation. Although such a change in heteroecious species has not yet been experimentally or directly observed, yet there are good grounds to believe that such is the method. Most of the 150 short-cycle species described in the North American Flora under *Teleutospora* and *Micropuccinia* have doubtless arisen from heteroecious species, and may eventually be so assigned. We are led to this belief, first, by finding short-cycle forms on aecial hosts, like *Puccinia andropogonis* in its short-cycle form on *Pentstemon* and *Comandra* and not on *Andropogon* or other grasses; and secondly by finding aeciospores and peridial cells in the sori of some short-cycle species, such as *Puccinia grindeliae* and *Uromyces scutellatus,* indicating that such species once had a distinct aecial stage.

Philosophizing upon the origin and phylogenetic development of the rusts has a number of important bearings. It develops a better understanding of the interrelationship of the different forms of rusts. It helps to point the way toward the completion of the life-cycle when only a single spore-form or generation is known. It tends to bring about a more consistent and stable classification. It enables the investigator of methods for economic control of the rusts to recognize whether he is working with the assistance of evolutionary processes or in opposition to them. The numerous theories regarding the course of rust development that have been advocated from time to time have in recent years been tested with a constantly wider knowledge of the less common rusts and also of a more detailed and searching study of the ones best known. Progress has been made all along the line.

If there is any one outstanding fact regarding the rusts that has received general acceptance beyond any shadow of doubt, and is confirmed

by experiment as well as observation, it is their strict parasitism. Repeated attempts to grow the rusts on artificial media, or even to accelerate the germination of the spores by using nutritive solutions, have met with uniform failure. But now word comes from Professor Fischer's laboratory in Bern that one rust has been discovered which shows a saprophytic characteristic. The spores of *Uromyces scillarum*, a not uncommon short-cycle rust on wild hyacinths in Southern Europe, have been found to germinate well in a decoction of horse dung, stewed plums, or garden soil, in fact much better than in water alone. This discovery should encourage further attempts to find some liaison in the rusts between parasitism and saprophytism.

There is one field of inquiry in which students of the rusts have made no progress, in fact have not even recognized the existence of the subject. "The discovery of things as they really are" is as important today as when Plato made the statement. What can be more reasonable and more necessary for the proper study of organisms of all grades from man to amoeba than the recognition of a distinction between normal, diseased, and deformed states. But in the study of the rusts all revelations of the microscope have generally been treated as normal, or variations of the normal, and the realms of pathology and teratology have been ignored or only vaguely hinted at, although in the study of the hosts there is no such confusion.

In the illustrations of germinating spores by the Tulasne brothers, Cornu, Carleton, Sappin-Trouffy, and other early and later botanists, all sorts of vagaries are figured from excessive elongation, unusual branching and abjunction of the promycelium, to misplaced and malformed spores. Such disturbances in normal behavior are largely traceable to the abnormal conditions under which germination is obtained. Rust spores germinate at their best for the most part in a saturated atmosphere, but the customary method of drop culture submerges them, and in this unnatural environment they show in their growth all sorts of ill effects. Such varied distortions are familiar objects, but the novelty of the subject lies in the interpretation. Carleton thought in 1886 that he had discovered a new method of germination, which might be utilized in classification. Clinton, in 1919, suggested that the rust derived an extended period for infection in some of the observed malformations. But whatever comments were made, there is no attempt to treat the matter from the standpoint of pathology. The recognition of disease in the host in connection with the rust is common, but never of disease in the rust itself.

When spores formed under normal conditions exhibit distortions in form or structure we have another situation which seems not to have been

evaluated correctly. The genus *Rostrupia* is a form of *Puccinia rubigovera* having more than two superposed cells in the teliospore. *Puccinia tomipara* is another form of the same species in which the teliospore is irregularly divided into a number of cells. The much-cited species *Puccinia vertisepta* is only a form with part of the teliospores having more or less oblique walls, a not unusual occurrence in many species. Such variations from the normal, which are properly disposed of under the caption teratology, having often been taken to indicate the course of development. Vuillemin, the able French mycologist, has made a study of the relationship of *Melampsora* and *Puccinia* based upon teratological material, and others have attempted to trace phylogeny by the same means. Such unusual spores are, however, only monstrosities and have little value in tracing either ontogeny or phylogeny, just as monstrosities among flowering plants have no taxonomic value. They may, however, become a hereditary feature, and be a characteristic of races, as in *Puccinia tomipara*.

Another field of inquiry that has not yet been explored is that of fossil rusts. About a half-dozen fossil forms, found in Europe, have been named, but all are of doubtless authenticity and add nothing substantial to our knowledge of early geological remains. There is every reason to believe, however, that valuable data will yet be derived when a suitable microscopical study of fossil structures is undertaken by persons equipped with detailed knowledge of mycelia, sori, and spores. There must also be a reasonable amount of taxonomic information. To know *Puccinia graminis* and rusts of that kind is not enough. Primitive rusts are likely to be of a different sort.

Having traversed by a series of saltations the ground covered by the announced topic of my discourse, starting in the pre-historical period, coming down to the present, and rebounding into the geological past, I now face about to see how much progress has really been made in rust study. Judging by comparison with the status of the subject at the beginning of the century there has been a marvelous advance, but in some directions more than in others. Morphology and life-histories have received much attention, but experimental development lags. We are just entering upon the highly promising study of heterothallism, mutations, and hybrids. The probable phylogeny of the rusts is still an open question. The nomenclature and classification of rusts are yet in a nebulous state, although progress has been made. Cytological methods have been utilized with great success, and yet only a few studies have followed the course of nuclear changes in pedigreed strains. The most conspicuous advance, and from the standpoint of the economist the most interesting, is that of specialization. I have not elaborated this part of the subject as much as its importance

apparently warrants, although I do not forget that I am speaking before a phytopathological society. My justification must lie in a premeditated intention to cover only the study of the rusts, and not that of the effect of the rusts upon their hosts, or of the protection of crops from their depredations. The great advances made in the application by physical science to practical requirements have never much outrun the theoretical and strictly scientific investigation of the underlying subject. I believe the same relationship will hold in mycology, and that the intimate study of the rusts, their history and development, will be an assistance in devising means for economic control.

TRANSMISSION OF THE VIRUS OF CURLY-TOP OF SUGAR BEETS THROUGH DIFFERENT SOLUTIONS

WALTER CARTER[1]

In a previous paper by the writer[2] a method is described by which the leafhopper *Eutettix tenellus* Baker can be fed on solutions. That curly-top can be transmitted to healthy beets by non-viruliferous leafhoppers which had previously fed on diseased beet juice was also reported. The question naturally arose as to whether, by the use of this method, the virus can be transmitted through solutions on which the insect could easily be induced to feed. In the present paper are given data obtained from preliminary studies made in this connection. At the time of writing the paper previously mentioned, positive results had been obtained by the use of three sources of the virus, (a) one drop of diseased beet juice in 40 cc. starch solution, (b) viruliferous leafhoppers macerated in the solutions, and (c) a very large number of leafhoppers from a badly diseased field, fed on the solutions from which the virus was later taken by non-viruliferous leafhoppers and transmitted to beet plants. These positive results, which are listed here, were obtained rather late in the season of 1926, and subsequent unsuccessful attempts to repeat the experiments may have been influenced by the poor conditions obtaining during the fall and winter under greenhouse conditions.

VARIOUS SOLUTIONS USED

During the spring and early summer of 1927 a large number of trials was made to transmit the disease through a number of solutions, but the results were all negative. To illustrate the range of materials used in the feeding solutions, some of them are listed (table 1).

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The painstaking assistance of Mr. Van E. Romney on the routine labor and greenhouse work in connection with these experiments is very gratefully acknowledged.

[2] Carter, Walter. A technic for use with homopterous vectors of plant diseases with especial reference to the sugar-beet leafhoppers, *Eutettix tenellus* (Baker). Jour. Agr. Res. 34: 449–452. 1927.

TABLE 1.—*Unsuccessful attempts to transmit sugar beet curly-top to non-viruliferous leafhoppers fed on solutions on which viruliferous hoppers had fed previously or to which were added macerated curly-top leaves or macerated viruliferous hoppers*

Date, 1927	Source of virus	Solution
March 28	Feeding of viruliferous leafhoppers	Diluted beet juice
April 15	do	Steam extracted beet juice diluted to amber color with addition of 1% sucrose and 0.2% gelatin
April 15	do	1% sucrose and 0.5% gelatin
April 19	do	1% sucrose, 0.5% gelatin with trace of the enzyme zymin
April 19	do	1% dextrin in distilled water
April 20	One drop juice from diseased beet	1% dextrin in distilled water
April 20	Feeding of viruliferous leafhoppers	1% sucrose and 0.5% gelatin
April 20	do	1% sucrose, 0.5% gelatin with trace of the enzyme emulsion
April 25	do	1% sucrose and 0.5% gelatin, plus 2% by volume liquid invertase and 1 drop acetic acid (dilute)
April 26	do	1% sucrose and 0.5% gelatin, with 2% by volume liquid invertase
April 27	Macerated diseased leaves	1% soluble starch
April 27	Macerated viruliferous leafhoppers	do
April 29	do	do
May 5	Feeding of viruliferous leafhoppers	1% sucrose
May 9	do	1% sucrose
July 3	Macerated diseased leafhoppers	1% soluble starch
August 1	do	1% sucrose
August 1	Feeding of viruliferous leafhoppers	Quassia extract and 1% sucrose
August 6	do	Quassia extract

EXPERIMENTS ON TRANSMISSION OF CURLY-TOP IN 1926

Transmission of Curly-top by using crushed leafhoppers as the source of the virus[3]

The feeding solution consisted of 12 crushed viruliferous leafhoppers, 0.4 per cent soluble starch, and 40 cc. distilled water. Non-viruliferous leafhoppers were caged on this solution August 24, 1926, removed and caged

[3] This transmission of curly-top using macerated viruliferous leafhoppers as the source of the virus was repeated later by Drs. Severin and Swezey, who used essentially the same technique in connection with another study. Acknowledgment is hereby made to these workers for their kind permission to refer to their confirmation of the writer's experiments.

on healthy beet plants August 31, and removed from the plants September 1. Curly-top symptoms appeared on two plants September 10 and on another September 14. One plant remained healthy. The same leafhoppers were caged on a second lot of four healthy beet plants for 24 hours on September 1. All these plants remained healthy. The leafhoppers were then caged on another lot of three healthy plants for 24 hours. These plants also remained healthy.

As a check on the preceding experiment, non-viruliferous leafhoppers were fed on a solution similar to that of the experiment except that crushed non-viruliferous leafhoppers were used. The non-viruliferous leafhoppers were transferred to healthy beet plants after feeding on the solution, but the plants remained healthy.

The fact is interesting that only plants in the first set of the experiment became diseased. The results are somewhat similar to those obtained when one set of viruliferous leafhoppers is transferred successively to a long series of plants. Under these conditions it often happens that the leafhoppers fail to transmit the disease to all the plants in the series.

Transmission of Curly-top by using the juice from diseased beets as the source of the virus

The feeding solution consisted of 1 drop of juice from a diseased beet in 40 cc. of a 1 per cent solution of sucrose. The solution was held for four days at 85° F., after which non-viruliferous leafhoppers were caged on it August 28, removed August 30, and caged on healthy beet plants the same day. Curly-top symptoms appeared 17 and 23 days later respectively.

Experiments in which maltose, dextrose, lactose, and raffinose were used in place of sucrose all gave negative results.

Since the transmission of curly-top by the use of juice from diseased beets as the source of the virus has already been recorded, the significance of this experiment lies in the fact that the virus was subject to high dilution.

Transmission of Curly-top by using, as the source of the virus, solutions on which viruliferous leafhoppers had fed

A feeding solution was used consisting of 0.2 gm. dextrose, 1.0 gm. maltose, 1.0 gm. sucrose in 250 cc. distilled water. A large number of viruliferous leafhoppers were caged with the solution for five days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. One plant out of five developed symptoms of curly-top 17 days later.

A second set of non-viruliferous leafhoppers was caged with the same solution and removed to healthy beets two days later. All these plants remained healthy.

Another feeding solution was then used consisting of the same sugars as for the preceding experiment with the addition of 1.0 gm. lactose and 0.5 gm. raffinose. Viruliferous leafhoppers were caged with this solution for five days. Non-viruliferous leafhoppers were then caged with the solution and removed to four healthy beet plants 2 days later, and allowed to feed for 24 hours. Three of these plants showed curly-top symptoms 12, 15, and 26 days later respectively.

The same leafhoppers were removed to a second set of healthy plants for another period of 24 hours. One plant showed curly-top symptoms 15 days later. Three other plants remained healthy.

The same leafhoppers were caged on a third set of healthy beet plants for 24 hours. One plant showed symptoms of curly-top 18 days later. One plant remained healthy.

A second lot of non-viruliferous leafhoppers was caged with the solution and removed to healthy beet plants after 2 days. Five hoppers were caged on each plant. One plant showed curly-top symptoms 51 days later. Three others remained healthy.

A third lot of non-viruliferous leafhoppers were caged with the solution and removed to healthy beet plants after 2 days. Three hoppers were caged on each plant. One plant showed symptoms of curly-top 70 days later and three plants remained healthy.

The period between exposure of the plant and the appearance of visible symptoms of disease ranged from 12 to 26 days for the first lot of non-viruliferous leafhoppers allowed to feed on the solution. The similar period for the second and third lots was abnormally prolonged, covering 51 and 70 days respectively.

Another feeding solution consisted of 1.0 gm. each of maltose, dextrose, and sucrose in 50 cc. distilled water. Viruliferous leafhoppers were caged with this solution for two days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. Two hoppers were caged on each plant. Two plants showed symptoms of curly-top. They had been abandoned as healthy after one month had elapsed since the plants were first exposed to the leafhoppers. The two diseased plants were not observed until after 39 days, but the incubation period was possibly a few days shorter than that.

Experiments run concurrently in which certain other sugars were used all gave negative results in curly-top transmission. These sugars were: Certo (fruit pectin), 1 per cent xylose, 4 per cent xylose, 1 per cent arabinose, 4 per cent arabinose. The solutions were prepared by mixing the sugars with 0.5 gm. potato starch in 250 cc. distilled water. Two lots of non-viruliferous leafhoppers were used in each experiment.

EXPERIMENTS IN CURLY-TOP TRANSMISSION DURING THE FALL OF 1927

A feeding solution of 1 per cent sucrose was used and a large number of leafhoppers from a badly affected beet field caged with it for six days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. Three hoppers were caged on each plant. One plant showed curly-top symptoms after 28 days and seven plants remained healthy. This experiment was then repeated. Two plants showed symptoms after 28 days and nine remained healthy.

Another feeding solution consisted of 1 per cent soluble starch in distilled water. Viruliferous leafhoppers were caged with the solution for five days. Non-viruliferous leafhoppers, after feeding on the solution for two days, transmitted the disease to one plant with an incubation period of 28 days. Seven plants remained healthy.

Two more experiments were made using the feeding solution consisting of 1 per cent sucrose in distilled water. Four positive cases of curly-top were obtained with incubation periods of 33 and 34 days. Seventeen plants remained healthy.

The experiments with the soluble-starch feeding solution were repeated. Three plants developed symptoms of curly-top after 33 days and 18 plants remained healthy. A second lot of non-viruliferous leafhoppers was caged with the solution and removed four days later to healthy beet plants. Five hoppers were caged with each plant. One plant became diseased 33 days later and five remained healthy.

The incubation periods of the disease obtained during the 1927 experiments were all considerably prolonged.

The non-viruliferous leafhoppers used in these experiments were taken from cages of reared material. These insects were checked for their freedom from disease by examining the healthy plants on which they were reared.

SUMMARY

1. Curly-top of sugar beets has been transmitted by leafhoppers which have fed on a suspension of crushed viruliferous leafhoppers in weak aqueous solutions of various sugars.

2. The disease has been transmitted by leafhoppers fed on diseased beet juice in a 1 per cent aqueous solution of sucrose.

3. The disease has also been transmitted by leafhoppers which had fed on a solution on which viruliferous leafhoppers had previously fed, the incubation periods in these instances, as a rule, being prolonged.

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FILTRATION EXPERIMENTS ON CURLY TOP OF SUGAR BEETS

HENRY H. P. SEVERIN AND OLIVE SWEZY

INTRODUCTION

It has been assumed by some investigators that curly top of the sugar beet differs from other plant virus diseases because direct artificial inoculation of healthy beets with diseased beet juice failed to produce symptoms of the disease. Smith and Boncquet (4) failed to infect healthy beets by inoculating them with juice from diseased plants. Severin (3) demonstrated later, however, that juice pressed from the leaves and roots of curly top beets and inoculated into the crown of healthy beets caused typical curly top symptoms in 9 of 100 beets. Carsner and Stahl (2) confirmed the results obtained by Severin. Since the method of artificial inoculation of healthy beets with diseased beet juice produced such a low percentage of infection, it was considered impractical for use in experiments for determining the possibility of filtering the causal agent of curly top.

METHODS OF INFECTING BEET LEAFHOPPERS

Beet leafhoppers, *Eutettix tenellus* (Baker), have been observed, on rare occasions, with their mouth-parts inserted in drops of the clear, viscid liquid which sometimes exudes from diseased beet leaves. It was assumed that non-infective adults might be infected by feeding them on diseased beet juice absorbed by pith, sponge, wick, or cotton. Non-infective hoppers which had been fed by each method transmitted curly top to healthy beets. These methods of infecting leafhoppers gave such a low percentage of curly top that they were abandoned in favor of the two methods given below.

Carter (1) described a method of feeding leafhoppers on liquids enclosed by a fish swim-bladder sack suspended in a celluloid cage containing the insects. This apparatus was used in a modified form in the work here reported. A small cylindrical cage 5¼ inches high and 4¾ inches in diameter was used, which was provided on one side with a glass window 3 inches high by 2½ inches wide. The other sides and top were covered with black sateen (Fig. 1). The leafhoppers, attracted by the light, would congregate on the sack or crawl up on the glass and in flying would often come to rest on the sack. This method was found most satisfactory in feeding the adults in the greenhouse. With the use of this feeding apparatus non-infective beet leafhoppers became infected and were able to transmit curly top to healthy beet seedlings.

FIG. 1. Small cylindrical cage used in feeding beet leafhoppers on solutions in a fish swim-bladder sack or culture dish. The sack was suspended in the cage by a thread passed through a small hole in the denim and fastened to a long nail. In the photograph the sack was hung on the outside to show its size. When the culture dish was used it was placed directly in front of the window.

An equipment for feeding the nymphs was also devised by Carter, which was used in a modified form for these experiments. This apparatus consists of a small culture or "Esmarch" dish (50 by 10 mm.) containing the food solution and placed in the cage described above. The dish was covered with a fish swim-bladder membrane (Fig. 1), the bubbles of air below the membrane being removed by brushing lightly over the top with a camel's hair brush. Elastic bands held the membrane in place over the outside of the dish. The feeding equipment was placed in front of the glass in the cage.

Feeding Period.—When the feeding period is prolonged under high temperatures in the greenhouse, a high mortality of the adults occurs. During 1926 a series of experiments was undertaken to determine the shortest period required for large numbers of bugs to feed on beet juice. It was found that males which had been fasted overnight in the greenhouse and placed in a cool room the next morning would feed on beet juice in large numbers in

from two to four hours after it was offered to them. Root juice immediately after extraction turns black, owing to oxidation processes, and in a few days bubbles of gas form as a result of fermentation. It is probable that the toxic nature of the food caused the high death rate found among the insects when carrying on these experiments.

The feeding period of the nymphs with the use of food solutions in culture dishes varied considerably according to the time of day at which the experiment was begun. If a feeding experiment was started in the morning the bugs were transferred to healthy beet seedlings after sundown; if the experiment was set up during the afternoon the hoppers were removed during the next morning.

If too many nymphs or adults were used in the feeding experiments, the membrane became sticky owing to the minute droplets of liquid oozing through the feeding punctures, and the hoppers adhered to the membrane like flies on tanglefoot paper. When an experiment was conducted over a period of several days under high temperatures in the greenhouse the membrane was changed daily to avoid this.

The number of adults used with the sack method of feeding was about 60. In many experiments a second lot of 60 males was fed on the solution at the end of the first day when the first batch was removed, followed, in some cases, by a third lot at the end of the second feeding. The same procedure was often adopted in the feeding experiments of the nymphs.

At the end of the feeding period each lot of nymphs or males was divided among three healthy beet seedlings enclosed in cages, where they remained for a period of five days. At the end of this time the bugs were removed and the beets were placed in quarantine or insect-proof chambers, where they remained for a period of two months if curly top symptoms did not develop within the usual period of ten days to two weeks.

A number of experiments were conducted to test the effect of sunlight and shade on the feeding of leafhoppers on the virus of curly top, using juice from the leaves and roots of diseased beets. Six cases of curly top developed in 29 beets inoculated by the leafhoppers in sunlight and seven in 24 beets inoculated in the shade of a room. It was therefore decided to conduct all of the following feeding experiments on the tables in the greenhouse, as the insects are more active in sunlight than in shade.

METHODS USED IN FILTERING BEET EXTRACT

In the preparation of juice from curly top beets for filtration the sugar beets were ground to a pulp in a sterilized food chopper. The pulp was then collected in folded cheesecloth in a sterilized dish and the juice pressed out by hand.

In the experiments with macerated leafhoppers the steam-extracted beet juice was prepared by placing beet roots, cut in pieces, in an autoclave for a period of about one hour. Sugar solutions were prepared with sterilized distilled water, and added to this extracted beet juice. In all experiments reported in this paper a 5 per cent solution was used.

In all the filtration experiments the liquids were centrifuged from one to two hours. As a general rule the solution was first passed through a large, coarse Berkefeld candle (8 by 1 inches) and refiltered through small, medium, or fine cylinders (2½ by ⅝ inches). With the use of this method little difficulty was experienced in filtering the juice from beet roots and culture media consisting of either a mixture of beet juice or steam-extracted beet juice and sugar solutions.

During 1927 a large number of filtrations were made using coarse (V), medium (N), and fine (W) Berkefeld candles or cylinders. The fine candles were tested with a small bacterium (*Bacillus prodigiosus*) so as to avoid the use of cracked or faulty candles with large pores.

FILTRATION OF ROOT JUICE

Experiments to determine the possibility of filtering the curly top virus were first undertaken by Severin and T. E. Rawlins during 1927. It was found that non-infective beet leafhoppers, after feeding on root juice, obtained from beets in an advanced stage of the disease and filtered through a coarse Berkefeld candle, transmitted curly top to healthy sugar beets.

During 1926 and 1927, root juice from 181 naturally infected sugar beets in an advanced stage of the disease, with wart-like protuberances on the lower surface of the leaves (Pl. XIV, B), was used in the filtration and control experiments. These badly diseased plants were selected in the beet fields of the Salinas and Sacramento valleys from May until October. Table 1 gives the results of the filtration of beet root juice with coarse, medium, and fine Berkefeld candles, also the unfiltered root juice used as a check or control.

As is shown in table 1, when non-infective beet leafhoppers fed on the filtered juice of diseased roots during the first day and then were placed on healthy beet seedlings, they transmitted curly top to 67.8 per cent of the plants used. Some experiments were continued for three successive days with a different lot of non-infective insects for each day. Those fed on the second and third days transmitted curly top to 26.6 and 7.6 per cent, respectively, of the healthy beet seedlings.

Filtration experiments were next conducted with root juice from beets showing the first visible foliage symptom of curly top, namely, the clearing or transparency of the minute veins (Pl. XIV, A, C). Infective beet leaf-

TABLE 1.—*The effect of inoculating sugar beets with curly top by means of leafhoppers fed on filtered juice from roots of sugar beets in an advanced stage of curly top*

Berkefeld candles used	Filtrate one day old		Filtrate two days old		Filtrate three days old	
	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Coarse	26	18	21	9	7	1
Medium	15	13	12	2	3	0
Fine	15	7	12	1	3	0
Total	56	38	45	12	13	1
Percentage infected		67.8		26.6		7.6
Unfiltered check	17	9	9	3	3	0
Percentage infected		52.9		33.3		0

hoppers were fed on 24 beets for a period of two weeks and then the juice was extracted from the roots. These beets were divided into two groups: number 1, consisting of beets with an average diameter of 1 inch, each infected with 25 male leafhoppers; and number 2, of beets with an average diameter of 2 inches, each infected with 50 male hoppers. Further details of these experiments are given in table 2.

Some of the experiments were continued for three successive days as in the preceding group. It is evident from tables 1 and 2 that a rapid inactivation of the virus begins after the first day, as is shown by the percentages of curly top obtained on the three successive days in both filtered and unfiltered root juice. This inactivation of the virus is probably associated with chemical changes in the root juice, oxidation, and fermentation processes.

FILTRATION OF MACERATED INFECTIVE BEET LEAFHOPPERS

In the filtration of macerated beet leafhoppers, approximately 1,000 or 2,000 adults were used in each experiment, about 16 hoppers being used for each cubic centimeter of culture medium in the first two experiments and about 33 leafhoppers per cubic centimeter in the last two experiments. The insects were transferred from diseased beets to an empty cage and were fasted for a period of 24 hours. This was done to insure evacuation of the food in the alimentary canal, thus avoiding the possible transmission of unchanged diseased beet juice.

In the preparation of macerated, infective beet leafhoppers for filtering, large numbers of adults were captured with a pipette (Fig. 2) and put in small specimen vials containing equal parts of steam-extracted healthy root juice and a 5 per cent sugar solution. The bottle was shaken so that the wings became wet and the hoppers were unable to fly. The bugs were then dumped into a small mortar and ground with a pestle large enough to fill the lower portion of the mortar. The macerated insects were then poured into test tubes containing the desired amount of beet juice and sugar solution.

The culture medium containing the macerated beet leafhoppers was centrifuged for one hour and then passed through a fine Berkefeld candle. In most of the experiments half of the filtrate was fed to 60 non-infective nymphs during the first day, while the remainder was incubated for one day at room temperature and fed to another lot of 60 non-infective nymphs during the second day. The results are given in table 3.

It is noteworthy that the greatest percentage of infected beets in table 3 occurs in those inoculated by hoppers fed on the second day, but the number of experiments is too small to draw any definite conclusions. It is evident from these experiments that a filterable stage of the virus occurs in the beet leafhopper.

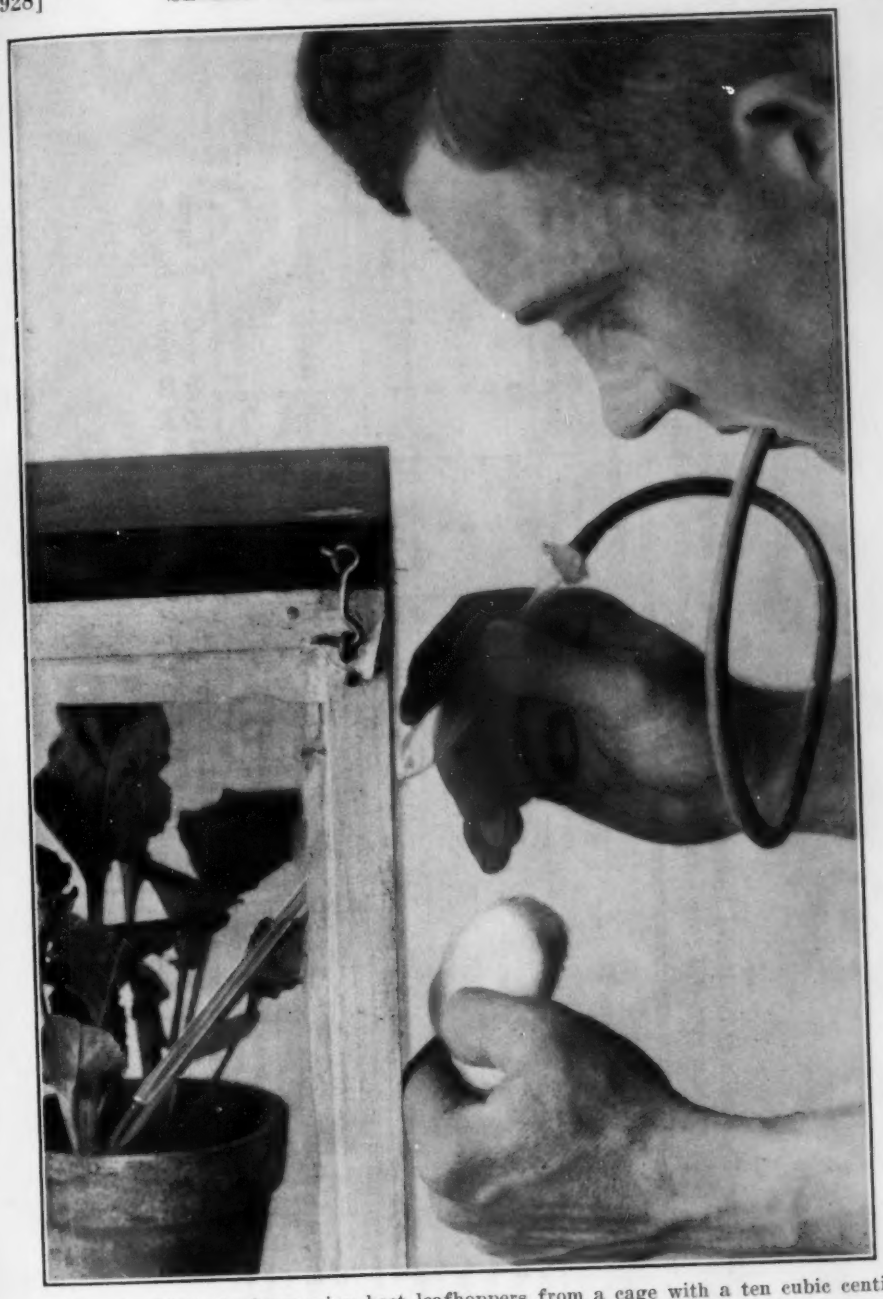

FIG. 2. Method of removing beet leafhoppers from a cage with a ten cubic centimeter pipette. By inhaling a breath of air through the rubber tube, the hoppers are drawn into the bulb of the pipette, and by exhaling the breath they are expelled from the pipette into a vial. A piece of silk bolting covers the opening between the pipette and rubber tubing.

TABLE 2.—*The results of inoculating healthy sugar beet seedlings with curly top by means of leafhoppers fed on a filtrate of juice extracted from beets on which infective leafhoppers had been fed for two weeks.*

Berkefeld candles used	Filtrate one day old		Filtrate two days old		Filtrate three days old	
	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Coarse[a]	3	3	3	1	3	0
Medium[a]	3	3	3	2	3	1
Fine[a]	3	1	3	0	3	0
Fine[b]	12	9	6	3	1	0
Total	21	16	15	6	10	1
Percentage infected		76.1		40.		10.
Unfiltered residue	6	3	3	0		
Percentage infected		50.		0		

[a] Beets in this group had an average diameter of 1 inch, and each one was infected by 25 male leafhoppers.

[b] Beets in this group had an average diameter of 2 inches and each one was infected by 50 male leafhoppers.

TABLE 3.—*The results of inoculating healthy sugar beet seedlings with curly top by means of nymphs fed on filtrates from centrifuged cultures of macerated leafhoppers,[a] beet juice, and sugar solution*

Kind of sugar used in culture medium	No. hoppers in 1 cc. of liquid	Fresh filtrate		Residue after centrifuging		Filtrate after incubation for one day	
		No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Beet, commercial	16	3	1	3	2	3	3
Cane, commercial	16	3	0	3	2	3	1
Sucrose, chemically pure	33	3	2	3	3	—	—
Saccharose, chemically pure	33	3	0	3	2	3	3
Total		12	3	12	9	9	7
Percentage infected			35.		75.		77.7

[a] The hoppers were collected from diseased beets and placed in an empty cage for a 24-hour fast before maceration.

SUMMARY

It has been found that non-infective beet leafhoppers when fed on root juice expressed from curly top beets in advanced and early stages of the disease and filtered through coarse, medium, and fine Berkefeld candles, transmitted curly top to healthy sugar beets. A rapid inactivation of the virus begins after the first day in both filtered and unfiltered root juice when exposed to the air through mouth-part punctures in the membrane.

Non-infective beet leafhoppers, after feeding on a culture medium in which infected leafhoppers were macerated, centrifuged, and filtered through a fine Berkefeld candle, transmitted curly top to healthy sugar beets. The infective beet leafhoppers were fasted for a period of 24 hours before maceration, so that the food in the digestive canal would be eliminated, thus avoiding the transmission of unchanged diseased beet juice.

It is evident from these experiments that a filterable stage of the virus of curly top occurs both in beets and the beet leafhopper which transmits it.

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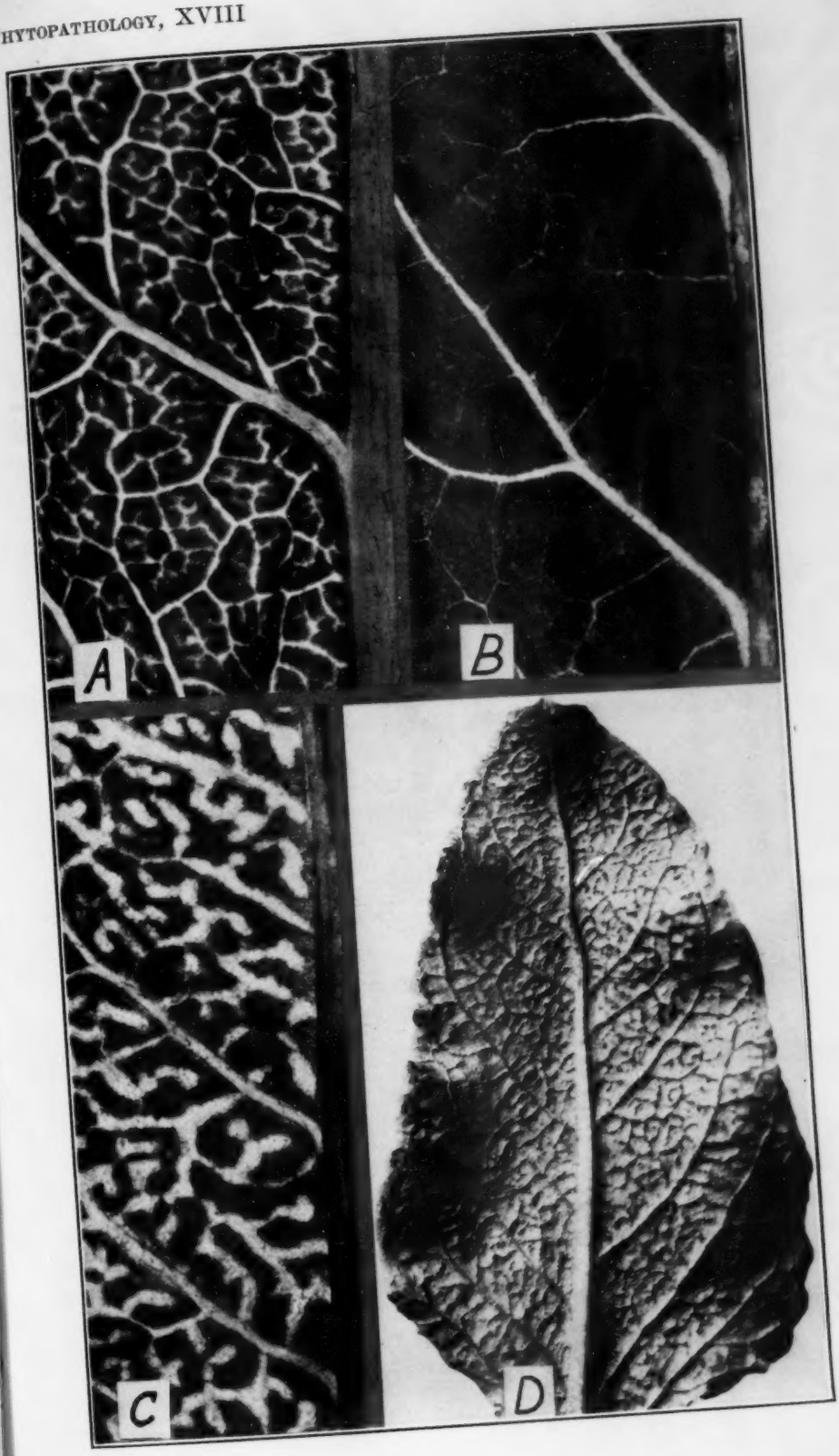
EXPLANATION OF PLATE XIV

A. Portion of youngest beet leaf affected with curly top showing early stage of transparent network of minute veins.

B. Portion of healthy beet leaf showing normal venation.

C. Later stage of transparent venation. The same magnification was used in figures A, B, C.

D. Beet leaf with small, wart-like elevations on the veins, which give the lower surface of the blade a roughened appearance.



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PRELIMINARY NOTE ON SOME SEROLOGICAL STUDIES OF ASPERGILLI[1]

TAKASHI MATSUMOTO

The object of the present investigation is to determine whether it is possible to differentiate any species of *Aspergillus* by means of serological methods. Most of the cultures of Aspergilli used in the present investigations were obtained from the National Collection of Type Cultures in London.

The morphology of the fungi was carefully studied, and they were arranged in groups according to the morphological relationships.

Group I.

Aspergillus niger, Strain A. Liquefies gelatin.

1. *Aspergillus niger* A, No. 1
2. do No. 2
3. do No. 3

Strain B. Does not liquefy gelatin, or only very slightly.

4. *Aspergillus niger* B, No. 1
5. do No. 2
6. do No. 3
7. *A. carbonarius*
8. *A. awamori*
9. *A. luchuensis*

Group II.

10. *Aspergillus fumigatus*, Nc. 1
11. do No. 2

Group III.

12. *Aspergillus glaucus*, No. 1
13. do No. 2
14. *A. herbariorum*
15. *A. amstelodami*

[1] This paper was presented at the annual meeting of the A. A. A. S. held at Nashville, Tennessee, 1927. The same article, accompanied by tables and more extensive discussions, will be published later.

Group IV.

16. *Aspergillus oryzae*
17. *A. parasiticus*

Group V.

18. *Aspergillus nidulans*
19. *A. versicolor*
20. *A. sydowi*

Group VI.

21. *Aspergillus ochraceus*
22. *A. tamarii*

Rabbits were inoculated, at intervals of 6 days, with increasing doses (5,000,000–160,000,000 spores) of the spore suspensions of the following six Aspergilli: (*1*) *A. niger A, No. 1,* (*2*) *A. niger B, No. 1,* (*3*) *A. carbonarius,* (*4*) *A. fumigatus, No. 1,* (*5*) *A. amstelodami,* (*6*) *A. glaucus.* The first two injections were given subcutaneously, and the subsequent eight inoculations made intravenously. The fungi were planted on ordinary agar slants and kept at 25° C. for one week. The spores were collected in sterilized physiological salt solution by scraping off with a platinum spud. These spore suspensions were centrifugalized, and supernatant fluids were discarded and replaced with sterilized physiological salt solution. The tubes containing those suspensions were left on a test-tube stand until the larger number of the clumps settled in the bottom of the tubes, and the supernatant spore suspensions were collected in separate tubes. The suspensions were heated for 45 minutes at 65° C. The number of spores in the inoculum was determined approximately by means of Thoma's counting chamber.

All the rabbits were bled from the marginal ear vein on the 9th day after the last inoculation. Sera were separated by pipetting from the clot after coagulation. They were always kept in the cold room, as the addition of a preservative is not advisable.

AGGLUTINATION TESTS

A rather extensive series of agglutination tests with homologous antigens as well as with heterologous antigens was made, but owing to the difficulty in making good spore emulsion the results were not satisfactory. A large number of the spores in normal sera settled within 30 to 60 minutes and formed a uniform layer on the bottom of the tubes in the same manner as in the immune sera; therefore no accurate reading as to the agglutinating

affinity could be satisfactorily made. In testing with anti-amstelodami serum against homologous antigens, I found a slight but distinct clumping of spores at dilutions up to 1:80 after one hour incubation at 57° C., while in the normal serum much slighter clumping was observed only at dilutions of 1:20 and 1:40. Nevertheless any cross tests proved to be unsatisfactory since the reading was rather unreliable in such a low titre serum as mentioned above.

PRECIPITATION TESTS

The precipitation tests were more satisfactory than the agglutination tests. A series of precipitation tests was carried out with filtrates of cultures grown in ordinary broth which had been incubated for 7 weeks at 25° C. Clear filtrates were easily obtained by passing the broth cultures through a filter-paper. If the filtrates were very acid, they were neutralized with KOH. All filtrates were diluted with physiological salt solution in the proportion of 1:1. For controls a salt solution and a normal serum were used.

As has been expected, the precipitation took place generally more extensively with the homologous antigens and next with the heterologous antigens of the same group. For instance, in the anti-niger A serum and the anti-amstelodami serum, a distinct precipitation was observed at the tested dilution of 1:40, and trace in 1:80; while with the heterologous antigens such precipitation, if present, was found only in 1:10 or up to 1:20.

In connection with this test it should be noticed that some slight precipitation was always found in the normal sera added to either one of the two filtrates of *Aspergillus niger* at the serum dilution of 1:10 and 1:20. No explanation as to this phenomenon can be properly made at this moment, as they are practically neutral. Moreover, no such precipitation was observed in the remaining filtrates.

It seemed advisable to repeat the same tests by using some extracts prepared from the organisms. Accordingly the following two techniques were employed.

1. Five grams of dried mycelium with spores, dried at 60° C., were thoroughly ground in a mortar and 100 cc. absolute alcohol added. They were extracted for 7 hours at boiling point in a reflux condenser. The extract was evaporated in order to be freed from alcohol, and subsequently saline slowly added. The solution was then passed through a filter-paper and finally through a Berkefeld filter.

2. Five grams of the dried organisms, dried in the same manner as described above, were thoroughly ground in a mortar and 100 cc. of 0.1 ethylene diamine solution added. They were put in an electric shaker for 4 hours, and then left on the laboratory bench overnight. The following

morning they were filtered through a filter paper and finally through a Berkefeld filter.

The results of the precipitation tests with these two extracts proved to be unsatisfactory, as the precipitation took place in no noticeable degree.

COMPLEMENT FIXATION TESTS

So far as my experiments are concerned, complement fixation tests appear to be most promising for differentiating our fungi, since the reactions are definitely observed in the higher dilutions of sera.

Technique. The reaction of the reagents and glasswares has been found to have an important bearing on the accuracy of the test. Acidity and alkalinity may give rise to false reaction which may be positive or negative according to the degree of acidity or alkalinity.

The reagents used in this experiment were as follows:

For fixation

0.5 cc. of immune sera at varied dilutions.
0.5 cc. of antigens in saline (1:200).
0.5 cc. of complement (1:10).

For hemolysis

0.5 cc. amboceptor, an exact concentration to be used is determined by means of titration.
0.5 cc. sheep red blood cells (1:20).

Immune sera were inactivated by heating for one-half hour at 56° C.

Antigens were prepared in the following manner. The fungi were planted on Czapek's solution agar and kept for one week at 25° C. The spores were collected in saline in the same manner as described above. To prevent the extraction from the media of substances that may render antigen non-specific, the spores were washed at least twice with saline. This was performed by centrifugalizing them and removing the supernatant fluids with a pipette. The spores were emulsified by addition of saline, in the proportion of 1 cc. of the spore mass to 200 cc. of the saline.

Freshly prepared complement of guinea pigs was used in a 10 per cent dilution made with saline.

Erythrocytes from sheep were used after they had been washed several times, and made up in a 5 per cent suspension with saline.

Amboceptor was obtained by successive inoculations of a rabbit with the sheep red-blood cells. The serum was heated at 56° C. for 30 minutes on three successive days. The balance of the hemolytic system was obtained by means of an amboceptor titration in the same method as generally ap-

plied for Wassermann test. The exact amount of amboceptor to be used was daily determined in this manner.

In the beginning of the work the fixation was accomplished by placing the test tubes in the incubator at 37° C. for one hour. Later I found that more reliable and complete reactions were obtained when the fixation was extended over night in the cold room. The following morning the sheep red-blood cells and amboceptor were added and incubated at 37° C. for one hour. The first reading was made after the incubation period of one hour, but the final reading was made after keeping the tubes in the cold room over night. For controls the following three tests were made: (*1*) saline instead of the immune sera; (*2*) saline instead of the spore suspensions; (*3*) normal sera instead of the immune sera.

In the majority of cases the immune sera were found to fix with the homologous antigens as well as with the heterologous antigens, but, as has been expected, the complement fixing power was always strongest when the immune sera were tested against the homologous antigens. For instance, in testing with the anti-niger A serum I obtained complete fixation up to 1:160, good partial fixation at 1:320, partial fixation at 1:640, and a trace in 1:1280. Practically the same extent of fixation was observed in the different strains of *Aspergillus niger,* thus showing no correlation between the serological behaviors and the biochemical characteristics as manifested by the liquefaction of gelatin. In this respect all the strains of *A. niger* studied were considered to be identical. In addition to this specific reaction the anti-niger A serum and anti-amstelodami serum have shown a distinct group reaction. In the anti-niger A serum *Aspergillus luchuensis* gave complete fixation at 1:160, good partial fixation at 1:320, and a trace at 1:640; *Aspergillus carbonarius* also completely inhibited hemolysis at 1:160, partially at 1:320, and a trace at 1:640; about the same result was obtained with *Aspergillus awamori.* In the anti-amstelodami serum, *Aspergillus glaucus* and *Aspergillus herbariorum* showed exactly the same complement fixing affinity; both gave complete fixation at 1:80, good partial fixation at 1:160, and partial at 1:320; whereas in the homologous antigen complete fixation was observed at 1:320, good partial fixation at 1:640, and partial fixation at 1:1280. Besides those reactions stated above these two sera, as well as the other sera, showed also some general group reaction against the remaining species, although the titre was very low with a few exceptions (mostly 1:10–1:40).

Anti-niger B serum, anti-fumigatus serum, and anti-glaucus serum also showed specific homologous reaction as well as group reaction to a certain extent, but titre was rather low as compared with the above two sera. This might be partly due to the characteristics of these fungi, but also due to the

individuality of the rabbits inoculated. A further investigation of this matter will be made later.

In view of the results obtained from the precipitation tests and the complement fixation tests, it may be considered that the sera contain in addition to specific homologous precipitins and complement fixing substances several groups of such substances which act on different antigens at the higher concentration. Therefore, if the titre of these sera is taken into consideration, these serological methods can be applied to identify or differentiate the fungi to a certain extent.

The writer is indebted to Dr. J. C. Ledingham and the other members of the staff of the Lister Institute of Preventive Medicine for many helpful suggestions.

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EFFECTS OF MOSAIC UPON THE CHLOROPHYLL CONTENT OF TOBACCO[1]

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Although the lighter green color is a marked pathological symptom of many mosaic diseases, there are but few studies of the chlorophyll of mosaic plants recorded in the literature. Dickson[2] has reported an apparent reduction in the chlorophyll content of the lighter green areas of mosaic tobacco plants and a greater-than-normal content in the darker areas. Elmer[3] has shown, in a quantitative manner, a similar condition in tobacco mosaic, and in addition has found a reduction of the green pigments in mosaic tomato leaves.

The purpose of this investigation was to compare the chlorophyll (a + b) content of the leaves of mosaic tobacco plants with that of the leaves of healthy plants grown under the same conditions. Normal and mosaic plants were compared, as to chlorophyll content, from the seedling stage to maturity. The effect of the mosaic disease upon the leaves at different positions on the stem was investigated. Determinations were also made of the effect of the disease upon older plants which had been infected for a comparatively short time. The total chlorophyll of a normal tobacco plant was compared with the amount of pigment in the entire leaf tissue of a mosaic plant.

The chlorophyll determinations were made upon Havana tobacco plants grown in the greenhouses of the Connecticut Agricultural Experiment Station during the period from November, 1926, to the following May. Most of the diseased material was obtained from plants which were infected in the seedling stage by rubbing the leaves with mosaic virus. In the case of smaller plants, from one to three leaves with the midribs removed were used for a single chlorophyll determination. With larger leaves, a rectangular area containing 60 sq. cm. was cut from one side of the leaf blade and used for chlorophyll extraction. Similar material was selected from normal and mosaic plants at the same time. The method of Willstätter as modified by Schertz[4] was followed in the extraction of the chlorophyll. Quantitative determinations of the saponified green pigments, chlorophyll

[1] Contribution from the Osborn Botanical Laboratory.

[2] Dickson, B. T. Studies concerning mosaic diseases. MacDonald College, Quebec, Tech. Bul. 2: 1922.

[3] Elmer, O. H. Transmissibility and pathological effects of the mosaic disease. Iowa State Coll. Agr. Res. Bul. 82: 1925.

[4] Schertz, F M. Bureau of Plant Industry, U. S. D. A., unpublished work.

a and *b*, were made with a colorimeter, using an aqueous solution of a weighed amount of purified chlorophyll, which had been saponified to chlorophyllins, as a standard for comparison.

Over one hundred chlorophyll determinations were made upon many plants during a period of 20 weeks after infection. These determinations were made consecutively over the entire period. The amount of chlorophyll in each determination was calculated upon a basis of 10 gms. of fresh leaves and upon 1 sq. dm. of leaf surface, although smaller amounts of leaf material were actually extracted.

A smaller amount of chlorophyll (a + b) in the mosaic plants, as compared with healthy material, was found at all stages of growth. The reduction was first apparent from 10 to 20 days after infection, when a significant difference of from 15 to 20 per cent was found. Later determinations showed that the chlorophyll content of mosaic plants was always less. The smallest reduction due to the mosaic disease was found at a period from 8 to 13 weeks after infection. At this time the chlorophyll content of the diseased material was only from 10 to 15 per cent less than that of normal plants. As the plants approached maturity an increase in the amount of chlorophyll in the normal plants was found. The determinations showed no corresponding increase in the mosaic plants.

TABLE 1.—*Chlorophyll content of mosaic and healthy tobacco plants*

Weeks after infection	Milligrams of chlorophyll				Mosaic / Normal	
	10 gm. of leaves		1 sq. dm. of leaves			
	mosaic	normal	mosaic	normal	weight	area
1.5–3	13.3	15.7	2.7	3.3	0.84	0.81
5–7	16.3	18.5	3.1	3.7	0.88	0.85
8–10	16.3	18.5	3.1	3.5	0.88	0.88
10–13	16.6	18.5	3.3	3.8	0.90	0.86
14–17	16.1	18.4	3.3	3.9	0.88	0.83
18–22	15.9	20.1	3.3	4.8	0.79	0.71

The chlorophyll contents of healthy and diseased plants at various periods after infection are given in table 1. Each figure represents the average of several determinations made in a given period of from two to four weeks. The chlorophyll contents given were determined from all the leaves of the youngest plants, while determinations upon larger plants were made upon the leaves half way up the stems. In the last two columns of the table are the chlorophyll content ratios of mosaic to healthy material, which were obtained by dividing the number of milligrams of green pigment found in a given amount of mosaic leaves by the chlorophyll content

of corresponding healthy material. Figure 1 shows the lower chlorophyll content of mosaic as compared with healthy plants, from the seedling stage up to maturity.

In comparing leaves from different parts of the stem of large, nearly mature, mosaic plants with similar healthy ones, it was found that the greatest reduction of chlorophyll occurred in the younger leaves. Leaves of healthy plants near the tops of the stems contained 20.4 mg. of chlorophyll per 10 m. of fresh leaves, whereas similar leaves from mosaic plants contained only 17.3 mg. Leaves farther down the stems were found to contain less chlorophyll, both in the diseased and normal plants. In the older leaves toward the base of the plants the reduction caused by the mosaic was less apparent.

Infection of older plants with mosaic virus caused a decrease in the chlorophyll content of the leaves that developed after infection, as compared

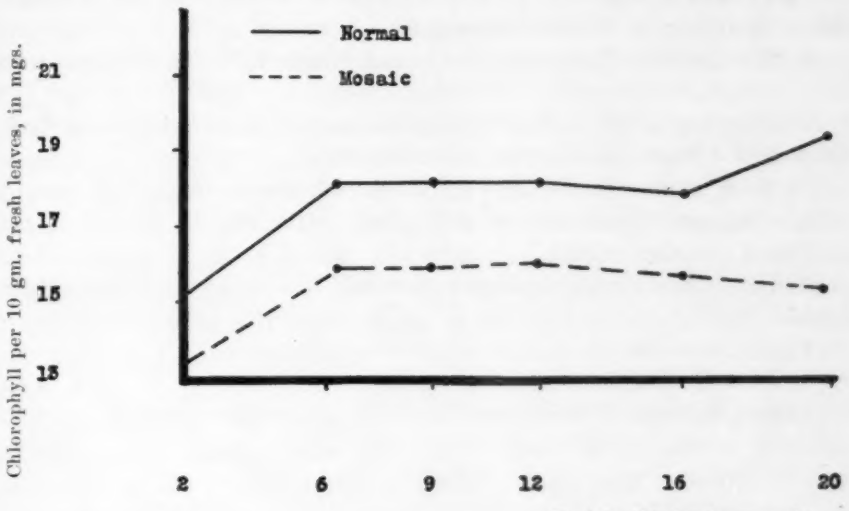

FIG. 1.—Chlorophyll content of tobacco plants throughout the growing period.

with healthy check plants. A slight loss of chlorophyll was found finally in the older leaves of the plants examined, although the older leaves showed no signs of mottling.

The total chlorophyll content of an entire mosaic plant was compared with the content of a corresponding healthy plant of the same age. The total area of these leaves on the normal plant was 89.7 sq. dm., as compared with 62.5 sq. dm. for the mosaic plant. The healthy plant was found to contain 359 mg. of chlorophyll, whereas the mosaic plant contained only 221

mg. In other words, the mosaic plant contained only 62 per cent as much chlorophyll as the normal plant. This difference was partly due to the somewhat stunted condition of the diseased plant and partly to the decrease of green pigment in a given amount of the mosaic material.

Aqueous solutions of the saponified chlorophylls from mosaic material have been observed under the colorimeter to be almost invariably of a yellowish green color as compared with the bluish green color of normal extracts. This change of color may be due to a greater proportion of chlorophyll *b*. Elmer[5] has found this latter pigment to be more abundant in mosaic extracts.

SUMMARY

1. The mosaic disease of tobacco reduces the chlorophyll (a + b) content below that of normal plants.

2. A lower-than-normal chlorophyll content is characteristic of mosaic tobacco plants at all stages of growth.

3. The amount of green pigment in any mosaic leaf of a tobacco plant is below normal.

4. The chlorophyll content of young tissues is somewhat more seriously affected by tobacco mosaic than older tissues.

5. Older tobacco plants, after infection with mosaic virus, were found to contain subnormal amounts of chlorophyll, especially in the leaves that developed after infection.

6. The total chlorophyll content of mosaic tobacco plants is seriously reduced.

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[5] Elmer, O. H. *l.c.*

AN ASCOMYCETOUS LEAF SPOT OF COWPEA

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Examination, during the course of our 1927 survey, of a field of cowpeas located near Huey, in Clinton County, Illinois, revealed a leaf spot of unknown cause. The spots on sample leaves taken from the field were found to bear perithecia, which, by their own characters, as well as the characters of their asci and spores, allied the leaf spot to similar diseases reported on alfalfa by Melchers (4) and F. R. Jones (2) as due to *Pleosphaerulina briosiana* Poll., on clover by Hopkins (1) as due to *Sphaerulina trifolii* Rostr., and on bur clover by Miles (5) as due to *Pseudoplea medicaginis* Miles. These repeated reports of similar fungi attacking and destroying the leaves of leguminous forage crops, together with the fact that the cowpea fungus possesses certain characteristics that differentiate it quite certainly from those previously noticed, lead us to give this brief account of a hitherto unknown cowpea disease.

The necrotic areas which appear as the result of the attack of this new fungus are exceedingly variable in size and seem to be influenced considerably by the resistance of the leaves, as indicated in the accompanying sketch (Fig. 1). The smallest spots, often not exceeding 2 mm. in diameter, are brown to tan, round to oval, and without a definite margin; spots from this size to 1 cm. wide are also brown to tan, round, and, in addition, nearly always have a distinct, rather wide, purple-tinted, dark margin; and the largest spots, reaching a width of 3 to 4 cm. or more, are of the same colors, except that the dark border is often very narrow.

Eventually the dead tissue in these spots becomes dry and friable and crumbles away, leaving large holes in the blade of the leaflet and deep semicircles in its margin. As the number of spots on a leaflet often is large, the destruction of leaf tissue is of considerable importance, and much damage to the plant must result. In the 5-acre field from which our samples and notes were taken, it was determined that 29 per cent of the plants bore this leaf spot, that on these plants 16 per cent of the leaves bore spots, and that as a result of the spots the surface of the leaves was reduced on an average, 13.5 per cent.

Even the smallest spots in the samples bore perithecia, and on the larger spots they were very numerous. Beneath the microscope they appear by reflected light to have burst the epidermis and to be protruding slightly, and when subjected to transmitted light they are seen to be mem-

FIG. 1. Cowpea leaves attacked by *Leptosphaerulina vignae*, and two spores of the fungus.

branous, yellow-brown to brown, and spherical to markedly applanate. The ostiole is dark and often carbonous. The asci, when still immature, are distinctly saccate, with a stubby foot and a very thick apical wall; but as they approach maturity they assume a definitely oblong outline, and the thickened apex becomes nearly as thin as the rest of the ascus-wall. The spores in immature asci are hyaline and show no order of arrangement, though their final form is foreshadowed by the presence of three or more lateral septa and, rarely, one or two longitudinal septa; but at maturity they are dark brown, always muriform, and surrounded by a thin, colorless envelope that is, apparently, quite distinct from the spore-wall. There are no paraphyses.

As Miles (5) has noted in his paper on the bur clover malady, fungi of this type have been considered by von Höhnel (7) as pseudo-sphaeriaceous; but Petrak (6), on the other hand, after more recent study, has contended that the species (*Pleosphaerulina briosiana* Poll.) upon which von Höhnel based his *Pseudoplea* is in all respects truly sphaeriaceous and is closely akin to *Pleospora*. Though certainly suggestive, the distinctions upon which von Höhnel based his mycological revisions have been so little approved that we hesitate to follow his generic segregation, preferring rather to locate the cowpea fungus as the second authentic species in McAlpine's (3, Figs. 105–107) genus *Leptosphaerulina*.

Leptosphaerulina vignae n. sp.

Foliicolous. Causing more or less extensive necrotic areas which are brown to tan with a dark, purple-tinted margin, extend through the leaf, become dry and friable and crumble away. Perithecia innate, becoming erumpent, developed in and occupying the epidermal and palisade layers, membranous, light brown or yellow-brown by transmitted light, spherical to markedly applanate, 75–100 μ in diameter; furnished with a dark to carbonaceous, round, protruding, and very slightly raised ostiole; upper half often seeming to bear setae, these being in reality conidiophores upon which are borne spores of an Alternaria type, apparently a conidial form. Asci few, 4 to 6, rarely 10, in a perithecium, at maturity oblong and short stalked, with a not very evident thickening of the apex, chiefly 74 μ long by 25 μ broad but varying widely, 8-spored. Ascospores 30–40 × 12–16 μ, arranged biseriately, brown at maturity, chiefly with 3 lateral septa, but occasionally with 5, each of the two intermediate compartments always parted by a single longitudinal septum (spores of 6 compartments often with 3 and 4 longitudinal septa), provided with a hyaline mucilaginous envelope 1–1.5 μ thick. Paraphyses absent.

On *Vigna sinensis* Hassk. Huey, Clinton County, Illinois. September 5, 1927. The type specimen, on which the above description is based, is deposited with the mycological collection of the Illinois State Natural History Survey under accession number 20937.

Aside from the economic importance which this fungus may have, we regard it as of rather unusual interest mycologically, in that it appears to combine characters from three closely related ascomycetous families. In lacking paraphyses, in the characteristic shape and lateral septation of its spores, and in the thickened apex of the ascus, it relates itself quite evidently to *Sphaerulina* and *Pseudosphaerulina* of the Mycosphaerellaceae; in the further septation of the spore, its coloration, and the presence of pseudolocular perithecial partitions (regarded by Petrak as immature asci, but, as it seems to us, more probably undeveloped or rudimentary paraphyses) it shows characteristics commonly encountered among the Pleosporaceae; and in the one character of a gelatinous, hyaline spore envelope it is suggestive of the Massariaceae.

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A CONSPECTUS OF NEEDLE RUSTS ON BALSAM FIRS IN NORTH AMERICA[1]

J. S. Boyce

The needle rusts of balsam firs form a group in which there has been considerable confusion. A study of herbarium specimens shows this, since collections under one specific name will in some instances have aecia on the current season's needles and in other cases on the needles of the previous season, or one year old. Field study has shown that the age of the needles on which aecia are found is of important diagnostic value, and not a single species is known in the Pacific Northwest with aecia normally on both the current season's and one-year-old needles, nor is any such occurrence elsewhere in North America reported in the literature. In fact, except for *Milesia pycnograndis* (Bell) Arth., the aecia of which occur on needles from two to six years old, the aecia of these rusts are practically confined to needles of one season. Some of these rusts are frequently encountered in the Pacific Northwest, and in order to get a clear picture the present knowledge of their relationships was tabulated and is presented here with the hope that it may be useful to others (Table 1).

TABLE 1.—*A conspectus of needle rusts on balsam firs*

Uredinia and Telia		Pycnia and Aecia		Age of needles with aecia	References to notes
Rust	Host	Rust	Abies host		
Calyptospora columnaris	Vaccinium spp.	*Peridermium columnare*	*amabilis* *balsamea* *concolor* *fraseri* *grandis* *lasiocarpa* *magnifica* *nobilis*	current season	(1)
Calyptospora sp. ?	*V. macrophyllum*	*P. ornamentale*	*concolor* *grandis* *lasiocarpa* *nobilis*	1 year	(2)
Melampsora humboldtiana	Salix spp.	*Caeoma americana*	*balsamea* *concolor* *grandis* *lasiocarpa*	current season	

[1] In the preparation of this paper, publications of Dr. J. H. Faull and his students on the needle rusts of Abies have been freely consulted. Acknowledgment is also made to Dr. Faull for his helpful suggestions regarding the manuscript.

TABLE 1.—(*Continued*)

Uredinia and Telia		Pycnia and Aecia		Age of needles with aecia	References to Notes
Rust	Host	Rust	Abies host		
Melampsorella elatina	Cerastium spp. Stellaria spp.	*Peridermium elatinum*	*amabilis balsamea concolor grandis lasiocarpa magnifica nobilis*	current season	(3)
Pucciniastrum abietichamaenerii	*Epilobium angustifolium* and other species	*P. pustulatum*	*amabilis arizonica balsamea concolor grandis lasiocarpa nobilis*	current season	(4)
P. epilobii	*E. adenocaulon* and other species	*P. pustulatum*	*balsamea*	current season	
P. myrtilli?	Vaccinium spp.	*Peridermium* sp. nov.	*amabilis*	1 year	(5)
Hyalopsora aspidiotus	*Phegopteris dryopteris*	*Peridermium pycnoconspicuum*	*balsamea*	2 years	
Milesia pycnograndis	*Polypodium vulgare*	*P. pycnogrande*	*balsamea*	2 to 8 years	
M. polystichi?	*Polystichum munitum*	*P. rugosum*	*amabilis grandis*	current season	(6)
M. kriegerina	*Dryopteris spinulosa*	*P. kriegerina*	*balsamea*	current season	(7)
M. marginalis?	*Dryopteris marginalis*	*P. marginalis*	*balsamea*	current season	(8)
Uredinopsis copelandi	*Athyrium cyclosorum, Felix bulbifera*	*P. balsameum*	*balsamea grandis lasiocarpa nobilis*	current season	
U. mirabilis	*Woodwardia areolata, Onoclea sensibilis*	*P. balsameum*	*balsamea*	current season	
U. osmundae	Osmunda spp.	*P. balsameum*	*balsamea*	current season	
U. phegopteridis	*Phegopteris dryopteris*	*P. balsameum*	*balsamea*	current season	
U. struthiopteridis	*Woodwardia virginica, Struthiopteris germanica*	*P. balsameum*	*balsamea*	current season	
U. macrosperma	*Pteridium aquilinum, P. pubescens*	*P. pseudobalsameum*	*amabilis grandis lasiocarpa nobilis venusta*	1 year	(9)

Note 1

Calyptospora columnaris causes witches' brooms on *Vaccinium*.

Note 2

Weir, J. R.: Observations on *Calyptospora columnaris* and *Peridermium ornamentale*. Mycologia **18**: 274–277, Pl. 34–35. 1926. Weir suggests in this paper that the telial form of *P. ornamentale* is *Calyptospora sp.*

Peridermium ornamentale at present is undoubtedly a composite species, because Peridermia with aecia on the current season's needles and others with aecia on the one-year-old needles are so named. While the rust is listed here as occurring on four species of *Abies*, this is done simply because three of these hosts have been recorded elsewhere. In the Pacific Northwest the writer has found *P. ornamentale* with the characteristic laterally-flattened aecia on *A. lasiocarpa* only, and field study indicates that it may be confined to this host. At Government Camp, Oregon, for example, where *A. lasiocarpa* has been found commonly infected for several seasons, the rust has never been found on *A. nobilis*, although this host is more abundant there than *A. lasiocarpa*. As far as the age of the attacked needles is concerned, *P. ornamentale* is reported here only as it occurs on the original host species, *A. lasiocarpa*.

Note 3

Melampsorella elatina causes witches' brooms with deciduous needles on *Abies*.

Note 4

In a paper read before the International Botanical Congress at Ithaca, New York, in August, 1926, Faull pointed out differences in the period of time from inoculation to emergence of the aecia between *Pucciniastrum abieti-chamaenerii* and *P. epilobii*. Since nothing is known concerning these two species on their aecial hosts in the West, the known Abies hosts for *Pucciniastrum pustulatum* have all been listed under *P. abieti-chamaenerii*, while only *Abies balsamea* has been given under *P. epilobii*.

Note 5

Boyce, J. S.: A possible alternate stage of *Pucciniastrum myrtilli* (Schum.) Arth. Phytopath. **18**: 623–625. 1928. In this paper it is suggested that the possible aecial stage of *P. myrtilli* is a Peridermium on *Abies amabilis*.

Note 6

Hotson, J. W.: Preliminary list of the Uredinales of Washington. Pub. Puget Sound Biol. Sta. Univ. Wash. **4**: 273–391. 1925. On page 293 Hotson

suggests the possible connection of *Milesia polystichi* and *Peridermium rugosum*.

Although the aecia of *P. rugosum* occur on the current season's needles, they appear in the fall or occasionally in the late summer, when the needles are morphologically about one year old.

Note 7

In North American Flora 7^{10}: 686. 1925, Arthur states that the aecia of *Milesia kriegerina* occur on leaves two or more years old. In a letter of April 7, 1927, referring to the aecia of this species, J. H. Faull wrote, "They are found always on the needles of the current season." In Scotland, when the writer was shown a needle rust on *Abies pectinata*, which from field study seemed to be *M. kriegerina*, the aecia were confined to the current season's needles.

Note 8

Milesia marginalis Faull and Watson was published by Faull in an abstract of a paper entitled, "Fern rusts I. The genus *Milesia*," which appeared in the Proceedings of the Royal Society of Canada, May Meeting, 1925. In North American Flora 7^{10}: 686. 1925, Arthur evidently includes *M. marginalis* with *M. kriegerina*, judging by the fern hosts given under the last-named species. In a letter of April 7, 1927, J. H. Faull wrote, "*M. kriegerina* and *M. marginalis* are entirely distinct species, distinguished from one another by several well-marked points."

The rare bristlecone fir (*Abies venusta* (Doug.) Koch) is a new host for *Uredinopsis macrosperma*. The collection was made by H. G. Lachmund on January 6, 1926, at an elevation of 1500 feet on Pick Creek, headwaters of the Big Sur River, Monterey Co., California. F. P. No. 40467.

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PHYTOPATHOLOGICAL NOTES

Tomato Yellows or Tomato Curly Top.—The name of this disease first appeared in the literature as "summer blight," then "yellow blight," next "western blight," and finally "western yellow blight." The word "tomato" has been omitted in some of the names of this disease or the list would be longer.

In a number of recent papers, Shapovalov suggests that the name "western yellow blight" be changed to "tomato yellows" and this change was approved by the Pacific Division of the American Phytopathological Society[1] but has not been acted upon by the committee on nomenclature.

A large number of other cultivated plants have been shown to be naturally infected with curly top; and this name should be retained, if possible, for all of these crops. Bean curly top has already appeared in the literature, also squash curly top.

If the name of this tomato disease is to be changed, then "tomato curly top" should be used rather than "tomato yellows," since "yellows" is already used with other insect-borne plant diseases. Yellows is also used with a considerable number of Fusarium diseases. The cultivated plants which are naturally infected with curly top would be segregated into a well defined group.

Kunkel[2] has experimentally transmitted aster yellows with the six-spotted leafhopper, *Cicadula sexnotata* (Fall.), to more than seventy species of plants in twenty-eight families. Kunkel[3] has experimentally transmitted this disease to spinach and has proved that aster yellows is identical with white-heart disease of lettuce and with a previously undescribed disease of buckwheat.

Aster yellows made its appearance in California a few years ago, and it has been demonstrated by transmission experiments with *Cicadula sexnotata* that celery and lettuce are naturally infected with this disease. Celery has been experimentally infected with curly top by the beet leafhopper, *Eutettix tenellus* (Baker), but has not been shown to be naturally infected with this disease up to the present time. Spinach has been proven to be naturally infected with curly top but not with yellows.

Confusion may arise if the term "yellows" is used for certain plants infected with curly top, especially if a plant is naturally infected with the

[1] Report of the eleventh annual meeting of the Pacific Division of the American Phytopathological Society. Phytopath. **17**: 745. 1927.

[2] Kunkel, L. O. Further studies on the host range of aster yellows (Abst.). Phytopath. **18**: 156. 1928.

[3] Kunkel, L. O. Studies on aster yellows. Amer. Jour. Bot. **13**: 646–705. 1926.

two diseases.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, Berkeley, California.

Grated Carrot Agar Favorable for Studies of Pythium.—It is sometimes difficult to obtain oospores from certain species of *Pythium* when they are cultured on the agars in common use in most laboratories. The writer has found this to be the case with a *Pythium* isolated from corn roots. A medium that has proved favorable for the production of oogonia and antheridia was prepared by adding grated carrots to water agar (17 grams of agar to one liter of water). In each test tube the grated carrot was placed to the depth of about 2.5 cm. and then approximately 10 cc. of melted water agar was added. The tubes were then plugged with cotton in the usual manner and autoclaved at a pressure of 15 lbs. for 30 minutes.

This medium was found to have the advantage of being sufficiently clear to permit examination of a plate under the microscope and yet to contain plant tissue without which some species of *Pythium* rarely fruit. Carrots were used because they are available throughout the year. Shredded fresh string beans were found equally satisfactory.—HELEN JOHANN, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture in cooperation with the Wisconsin Agricultural Experiment Station.

Disinfectants in Fire Blight Eradication Work.—Reimer[1], in Oregon, found that mercuric chloride was not uniformly effective as a disinfectant in fire blight eradication work, and adopted a mixture of this material with mercuric cyanide (1–500 each in water). Day[2], working under arid conditions in California, concluded that glycerine (three parts to one part of water) when added to the above mixture greatly improved its effectiveness. The writer has tested these materials in several experiments at Ithaca, New York. On cut twigs of vigorous apple trees mercuric chloride (1–500) injured the tissues to an average distance of 0.14 cm. from the wound, while mercuric cyanide (1–500) alone or in mixtures killed back the terminals 1.7 to 2.2 cm. One hundred and seventy twigs were treated in these tests.

Similar treatments were performed on wounds made with a cork-borer on trunks and branches of pear and apple trees. All of these wounds were inoculated with the fire blight organism from bouillon cultures. Mercuric chloride caused no measurable injury in these tests. In 2 of 35 wounds treated with this material, fire blight developed. Mercuric cyanide alone and in mixtures killed the bark to a distance of 0.2 to 0.4 cm. from the

[1] REIMER, F. C. A new disinfectant for pear blight. Calif. St. Comm. Hort. Mon. Bul. 7: 562–565. 1918.

[2] DAY, L. H. Experiments in control of cankers of pear blight. Phytopath. 14: 478–480. 1924.

margin of the wound, but not a single canker developed from the 59 wounds treated. In 6 of 24 inoculated but untreated wounds blight developed. The penetration of mercuric cyanide was apparently not appreciably increased by the addition of mercuric chloride or glycerine.

Mercuric chloride alone has been used in this state with apparently satisfactory results for many years. However in view of the available evidence and of the writer's own experience in practical eradication work, the mixture of mercuric chloride and mercuric cyanide in water is preferred for New York conditions until further data are available.—FRANK L. HOWARD, Department of Plant Pathology, Cornell University, Ithaca, New York.

The effect of washing the seed on infection of wheat by stinking smut.—During the course of some seed disinfection tests, it occurred to the writer to determine the effect of washing on the infection of wheat by stinking smut, *Tilletia levis*. Seed of an unknown variety, probably Fulcaster, was used. It was quite black with spores and contained 18 per cent of unbroken galls. A portion of the seed was placed in water and the floating trash and galls were skimmed off. It was then placed in a sieve under a stream of water from a tap, where it was washed for one hour. To the unaided eye the seeds were clean and bright after washing, but when viewed through the microscope many spores still remained on the brush ends of all seeds examined. Three brands of copper carbonate, designated as A, B, and C, were included in the test, also Bayer Dust and Semesan applied as a dust. The seed used for the dust treatments and for one of the check lots was hand cleaned before treatment, all galls being removed. The dusts were applied in excess and the excess removed with a sieve. An additional check lot from which the galls were not removed was also provided. The lots were seeded by hand on October 13, 1925, each occupying a single row 66 feet in length. They were harvested at maturity in June, 1926. Data on sound and infected heads are recorded in table 1. In view of the intensity of infection in the check lots the results obtained by washing appear very striking. The washing process gave as satisfactory control as the average of the dust disinfectants.

A second test of the effects of washing was made in the fall of 1926. The seed used was also quite black with spores of stinking smut and contained a number of unbroken galls which were removed by skimming. The lot subjected to washing was placed under the tap as in the tests of the preceding year and samples of 30 grams were removed after intervals of 15, 30, 45, 60, and 120 minutes. Two check lots, as well as a number of other treatments which will not be recorded here, were also provided. One check lot was

TABLE 1.—*The effect of washing and other seed treatments on infection of wheat by stinking smut*

Treatment	No. heads	No. infected	Percentage infected
Check, uncleaned	2270	1233	44.52
Check, cleaned	2040	800	39.22
Semesan, dust	1454	40	2.75
Bayer Dust	1523	34	2.23
Washed one hour	1602	13	0.81
Copper carbonate, Lot A	2000	4	0.20
Copper carbonate, Lot B	1613	2	0.12
Copper carbonate, Lot C	863	1	0.12

soaked 60 minutes in standing water, the galls being skimmed off, while the other was seeded dry without treatment. The lots were seeded by hand in 10-foot rows on October 2, 1926. Data obtained at harvest in 1927 are recorded in table 2. A marked reduction in percentage of infected heads in the washed lots will again be noted. Washing of 15 minutes reduced infection from 5.84 per cent in the dry check to 0.9 per cent, and additional washing resulted in further reduction.

TABLE 2.—*The effect of washing for stated periods on infection of wheat by stinking smut*

Treatment	No. heads	No. infected	Percentage infected
Check, soaked one hour	386	25	6.48
Check, dry	428	25	5.84
Washed 15 minutes	442	4	0.90
Washed 30 minutes	423	2	0.47
Washed 45 minutes	385	2	0.52
Washed 60 minutes	382	0	0.00
Washed 120 minutes	354	1	0.28

It seems evident that the reduced infection is to be attributed to the mechanical removal of spores by the stream of water. It could not be due to the action of water alone, since the lot soaked for 60 minutes in still water showed no reduction in infection. Such an interpretation is strengthened by the studies of Heald[1] on the relation of the spore load to infection. In these studies a minimum dosage of 0.5 grams of spores to 100 grams of seed was required to produce the maximum percentage of infected heads. A

[1] HEALD, F. D. The relation of spore load to the per cent of stinking smut appearing in the crop. Phytopath. **11**: 270–278. 1921.

very considerable reduction of the spore load was accomplished by the washing process.—F. D. FROMME, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

Prize Offered by the State of Rio de Janeiro, Brazil. The State of Rio de Janeiro, Brazil, has announced the offer of a prize of 100:000$ (approximately $1,200) to the scientist, either Brazilian or foreign, who prior to December 31, 1928, determines in an accurate and scientific manner the etiology of sugar cane mosaic and an effective method of combatting it, and presents the best thesis on this subject, this thesis to be published for the information of the interested public. Further information can be had from Dr. Eurico Teixeira Leite, Director, Instituto Fomento e Economia Agricola, Ministerio da Agricultura, Rio de Janeiro, Brazil.

BOOK REVIEW

Die Krankheiten der Obstbäume und Obststräucher. By E. Ewert. 2te neubearbeitete Auflage mit 63 Textabbildungen. 145 pp. Berlin. Paul Parey, 1926.

Professor Dr. Ewert has been the Director of the Botanical Experiment Station of the State College of Pomology and Horticulture at Proskau in Silesia, Germany.

In the first chapter a very short and popular survey of the system of fungi is given; some words are added on environmental factors and animal parasites.

The second chapter deals with disease control through fungicides, insecticides, insect traps, protection of birds and useful insects, and wound-cure.

Diseases common to all kinds of fruit trees are dealt with in the third chapter. It includes a discussion of conditions unfavorable for root development, root fungi, water rats (*Arvicola amphibius*) attacking the roots; frost, hail, hares, wood caterpillars, wood beetles, moss and lichens attacking or living on the stems and branches; dryness, silver leaf, chlorosis, caterpillars and beetles attacking the leaves and twigs; frost, caterpillars attacking the flowers; fruitdrop, cracking, sunscald, frost, and insect injury of fruits.

A similar arrangement is followed in the following special chapters: 4, on apple diseases; 5, on pear diseases; 6, on cherry diseases; 7, on plum diseases; 8, on peach diseases; 9, on apricot diseases; 10, on medlar diseases; 11, on quince diseases; 12, on currant diseases; and 13, on gooseberry diseases.

Besides some illustrations derived from Sorauer's *Handbuch der Pflanzenkrankheiten*, Ewert's booklet contains some good original photographs, *e.g.*, the results obtained when currants are sprayed with bordeaux mixture as a protection against *Gloeosporium ribis*.

We see here, as in many German books, that discoveries made in other countries are not given the attention they deserve; the fact that silver leaf is caused by *Stereum purpureum* is not fully acknowledged. That *Nectria galligena* enters through leaf scars and scab wounds is not mentioned. Very valuable are the author's extensive observations on susceptibility of varieties. *Pinus cembra*, according to the author, is not a host for *Cronartium ribicola*. Carbolineum, which in Holland is one of the leading winter sprays, is not recommended in Germany on account of the inconstant composition. A spray calendar is not added.

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